

FISHERY INVESTIGATIONS

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Studies on the food value of
nineteen genera of algae to juvenile
bivalves of the genera *Ostrea*,
Crassostrea, *Mercenaria* and *Mytilus*

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MINISTRY OF AGRICULTURE AND ANIMAL INDUSTRY
FISHERY INVESTIGATIONS

Studies on the food value of
mullet (Mugil cephalus) in juvenile
divisions of the range of
Chesapeake, Maryland and Virginia

W. E. JAMES

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ABSTRACT

An account is given of the growth obtained by feeding juvenile oysters and clams on a variety of uni-algal diets. Various concentrations of food were used. Marked differences were found in the food value of different algal species, with *Dicrateria*, *Isochrysis*, *Skeletonema*, *Tetraselmis*, *Chaetoceros* and *Monochrysis* giving good results with oysters. The results with clams were similar, except that *Monochrysis* and *Dicrateria* were not so prominently good. Feeding at the correct concentration was particularly important for oysters but less so for clams.

INTRODUCTION

The food requirements of the larvae of some species of marine bivalves have received considerable attention because of these species' commercial value (for recent reviews see Walne 1964, 1965; Loosanoff and Davis 1963), but the requirements of the stages subsequent to metamorphosis have been little studied. Some observations have been made on the nature of the particles ingested (e.g. Savage 1925), but it is known for larvae that this is not a good guide to food value; many species of algae are ingested but not utilised. The same phenomenon probably occurs in the adults. Nelson (1947) commented: "Even after half a century of research on the oyster we are still not in a position to say with certainty just what this mollusc can or cannot use as food". Wilbur and Owen (1964) more recently, commenting on molluscs in general, said: "In fact, we do not know the organisms or materials serving as major nutritional sources for most species".

This paper presents the results of feeding a number of species of uni-cellular algae to the juveniles of several species of bivalve molluscs. The methods adopted were intended to give a general picture of the value of algae from several systematic groups; it was not intended to go into details of conversion efficiency, or to test the suitability of a food over a long period of time, or to examine the value of a food in supporting reproduction. Similarly no part of the work was done with bacteria-free cultures, since their use poses many practical problems and would have made it impossible to carry out such extensive trials. The fact that foods are likely to be used in commercial conditions is a strong argument for studying the value of algae cultured with a natural bacterial flora.

METHODS

The procedure used to assess the food value of an algal species was to grow samples of the juvenile bivalves in several concentrations of the food in filtered sea water for a period of about three weeks. This method ensured that at least one of the tests was at a favourable concentration of the food. With each series of experiments a control was also included, using either *Isochrysis* or *Tetraselmis* as a food. In some cases the food being tested was also fed in sea water which had been coarsely filtered (referred to in the text as unfiltered). This, unlike the filtered water, usually supported growth and the test showed whether the experimental food increased the natural food value of the water. This procedure helped to ensure that the poor result of an algal species was not due to the absence of very small quantities of some necessary compound, and also served as a check on whether a particular food inhibited growth.

Three times a week the water was changed, the animals were washed and replaced, and the necessary volume of algal culture was added. At intervals during the experiment the animals were weighed or measured. In most cases each food was tested at least twice, at different times, and with animals of different parentage.

ANIMALS

Juvenile animals were used in all experiments; because of their size the volume of water and the quantities of algal culture required were reduced compared with those which would be required for adults, and there were no complications due to the production of gonadial material.

All the tests, with the exception of a few with *Mytilus edulis*, were made with stock reared in the Conway hatchery by a standard technique (Walne 1966). Not only was the stock for each experiment of known age, but as they were also siblings this reduced the variation in growth rate within a sample. The great majority of experiments were made with the European flat oyster, *Ostrea edulis*, and the American hard clam, *Mercenaria mercenaria*. All the species used are listed, with the origin of the parent stock, in Table 1.

The experiments with oysters (*O. edulis*, *Crassostrea gigas*) typically started within one to three days of the metamorphosis of the larvae. To obtain juveniles in a manner in which they could be conveniently handled, frosted glass plates 9 cm × 9 cm were placed on the bottom of the larval culture vessels when settlement commenced. To encourage the spat to settle on the plates, these were often first painted with an aqueous extract of oyster meat which was allowed to dry before being offered to the mature larvae. Usually sufficient spat became attached within 48 hours.

Table 1 The animal species used in this study and the origin of the breeding stock

Species	Origin of breeding stock
<i>Crassostrea angulata</i> Lamark	Portugal and subsequently relaid in the River Crouch before moving to Conway
<i>Crassostrea gigas</i> Thunberg	British Columbia, Canada
<i>Mercenaria (Venus) mercenaria</i> L.	Southampton Water, England
<i>Mytilus edulis</i> L.	Not bred; wild stock from the River Conway
<i>Ostrea edulis</i> L.	Various fisheries in England, and hatchery bred in Conway
<i>Ostrea lutaria</i> Hutton	Fouveaux Strait, New Zealand

The experiments with *Ostrea lutaria* differed because in this species the larvae are planktonic only for an hour or so (Millar and Hollis 1963). Here the plates were hung for a few hours in a tank holding the breeding stock when a liberation of larvae had been observed.

With all species of oyster, the plates carrying recently metamorphosed larvae were usually placed for a day or two in filtered or unfiltered water enriched with *Isochrysis* or *Tetraselmis*; this helped to ensure that the juveniles in a batch were healthy and growing normally after metamorphosis. Either at this stage or within a few days after the start of the experiment the number of juveniles on the plates was, if necessary, reduced. The aim was to have about twenty individuals on a plate.

Competition for space on the frosted glass plates was not a serious problem until the oysters grew so large that the shells started to overlap. This was confirmed in one experiment using five plates. The juvenile *Ostrea edulis* were reduced to different numbers on each plate and on any one the animals were approximately equidistant. They were all kept in the same tank of unfiltered sea water enriched with 75 cells of *Isochrysis* per μl . After 14 days the mean sizes were as follows:

Number of oysters per plate:	324	,	196	,	81	,	36	,	9
Mean shell length (mm):	3.3	,	2.8	,	2.9	,	3.3	,	3.2

The clam, *Mercenaria mercenaria*, does not form a permanent attachment at metamorphosis, nor do the young stages grow as rapidly as oysters. It was therefore practicable to use clams of varying ages from a few weeks to about a year. This corresponds to a size range of 0.6 to 7 mm. The stock for an experiment was obtained by selecting those individuals in a narrow size range from a batch of siblings.

In this study 'growth' has usually been measured as an increase in shell size. Oysters were measured along the antero-posterior axis, using a stereoscopic microscope fitted with an eyepiece micrometer. This was marked with 100 divisions, and a combination of objectives gave 1 mm as equivalent to 12, 25 or 48 divisions. During measurement the frosted glass plate was laid on a piece of gridded transparent plastic. Since the plate was always the same way up, the position of each animal could be recorded. It was not possible to study the change in weight during growth, but some measurements were made of the dry weight at the end of the experiment. The upper, or right valve, could readily be removed and weighed to give a measure of the shell growth. Dry meat weights were obtained by removing the meats and drying at 100°C for 24 hours on either tared cover slips or pieces of tared cigarette paper. An index of the condition of the oysters is obtained by calculating the dry shell weight:dry meat weight ratio.

Clams were also measured along the antero-posterior axis or, more frequently, weighed. Since the individuals of a batch were indistinguishable, unless the shell was marked with paint, it was found quick and convenient to weigh a whole batch together after blotting dry on a tissue. The relation between the mean length of a batch and the live weight was very constant and was not influenced by the nature of the food or the rate of growth. During the study 233 batches of 10 to 25 animals were weighed and measured, and the regression formulae for relating length and weight were: $W = 2.983L - 0.531$ and $L = 0.333W + 0.181$, where $W = \log_{10}$ weight in mg and $L = \log_{10}$ length in mm. The correlation coefficient was 0.9965.

Table 2 The origin and cell size of the algae used in this study. The packed cell volume was found by centrifuging samples of culture, and the median cell volume was estimated from the distribution of cell sizes obtained with a Coulter counter. Where the median cell volume is shown in parentheses, it has been estimated from the packed cell volume, using Figure 2. Where no sizes are shown, see text

Species	Origin of strain	Packed cell volume of 10 ⁶ cells (μ l)	Median cell volume (μ^3)
HAPTHOPHYCEAE			
<i>Dicrateria inornata</i> Parke	Plymouth B	0.03	21
<i>Isochrysis galbana</i> Parke	Plymouth and Burnham-on-Crouch	0.124	57
<i>Cricosphaera carterae</i> (Braarud and Fagerland) Braarud	Plymouth 156 and 181	5.10	900
CHRYSTOPHYCEAE			
<i>Monochrysis lutherii</i> Droop	Droop, Millport 60	—	32
<i>Pavlova gyrans</i> Butcher	Milford, U.S.A. (originally Butcher, Burnham-on-Crouch)	—	43
PRASINOPHYCEAE			
<i>Micromonas pusilla</i> (Butch.) Manton et Parke = <i>Chromulina pusilla</i> (Butch.)	Butcher, Burnham-on-Crouch	0.006	4.5
<i>Micromonas squamata</i> Manton et Parke	Plymouth 189	—	36
<i>Tetraselmis suecica</i> (Kyllin) Butch.	Butcher, Burnham-on-Crouch	0.734	335
<i>T. tetrahele</i> (G. S. West) Butch.	Butcher, Burnham-on-Crouch	0.45	(250)
<i>T. inconspicua</i> Butch.	Butcher, Burnham-on-Crouch	1.12	(580)
<i>T. chui</i> E. G. Prings, ex Butch.	Cambridge LB 8/6	—	520
<i>Pyramimonas grossii</i> Parke	Plymouth 78	—	—
CHLOROPHYCEAE			
<i>Dunaliella tertiolecta</i> Butch.	Plymouth 83	0.46	300
<i>D. eichloria</i> Lerche	Milford, U.S.A.	—	208
<i>Chlamydomonas coccoides</i> Butch.	Butcher, Burnham-on-Crouch	0.30	150
<i>Chlorella stigmatophora</i> Butch.	Butcher, Burnham-on-Crouch	—	27
<i>Chlorella autotrophica</i> , Shihira and Krauss	Chlorella 580; Milford, U.S.A.	—	—
<i>Nannochloris atomus</i> Butch.	Butcher, Burnham-on-Crouch	0.008	5.2
<i>Brachiomonas sub-marina</i> Bohl	Butcher, Burnham-on-Crouch	—	260
XANTHOPHYCEAE			
<i>Olisthodiscus</i> sp.	Plymouth 239	3.18	(1 500)
CRYPTOPHYCEAE			
<i>Cryptomonas</i> sp.	Cryptomonad 5A, Milford, U.S.A.	—	46
BACILLARIOPHYCEAE			
<i>Phaeodactylum tricornutum</i> Bohlin	Plymouth	0.14	60
<i>Thalassiosira fluviatilis</i> Hustedt	Guillard 'Actin' Woods Hole	—	—
<i>Skeletonema costatum</i> (Greville) Cleve	Droop 73, Millport	—	—
<i>Chaetoceros calcitrans</i> (Paulsen) Takano	Imai, Sendai	—	50

SEA WATER

The sea water was drawn from the Conway Estuary, and the system of heating, distribution and filtration has been described in detail in Walne (1966). The salinity varied between 28‰ and 34‰. The warmed sea water was filtered and sterilised by successively passing through a sand filter, a ceramic filter and finally an ultra-violet steriliser. Towards the end of the work reported in this paper the sand and ceramic filters were replaced by a plate filter using diatomaceous earth as a filtering medium. In both systems, the water was filtered at about 10 litres per minute.

Part of the success of experiments of the type reported in this paper depends on the satisfactory treatment of the sea water, and observations have been made on the inability of the filtered water to support growth, and also on the number of particles present in it. Examples of the small amount of growth in the filtered water are given in Figure 7 and App. Tables 4, 5 and 20. The particle content was determined by counting samples with a model A Coulter counter; with this instrument it is possible to obtain an estimate of both the number of particles and their size, in terms of volume (Sheldon and Parsons 1966). About 50 samples were analysed at intervals during 1966 and the results are set out in Figure 1 as histograms in terms of numbers of particles larger than $17\mu^3$ per ml; this is equivalent to a rather small flagellate cell (see Table 2). Most of the seawater samples contained 10 000 to 20 000 particles per ml and this was reduced to less than 4 000, and often substantially less, by filtration.

Unfiltered water, sometimes referred to as 'raw' sea water, was used in some experiments. This was obtained from the laboratory supply before it entered the main filtration system. Before use it was coarsely filtered through a nylon screen with a mesh size of 64μ which removed the larger competing organisms such as copepods and debris of various sorts, but allowed all the smaller phytoplankton to pass through.

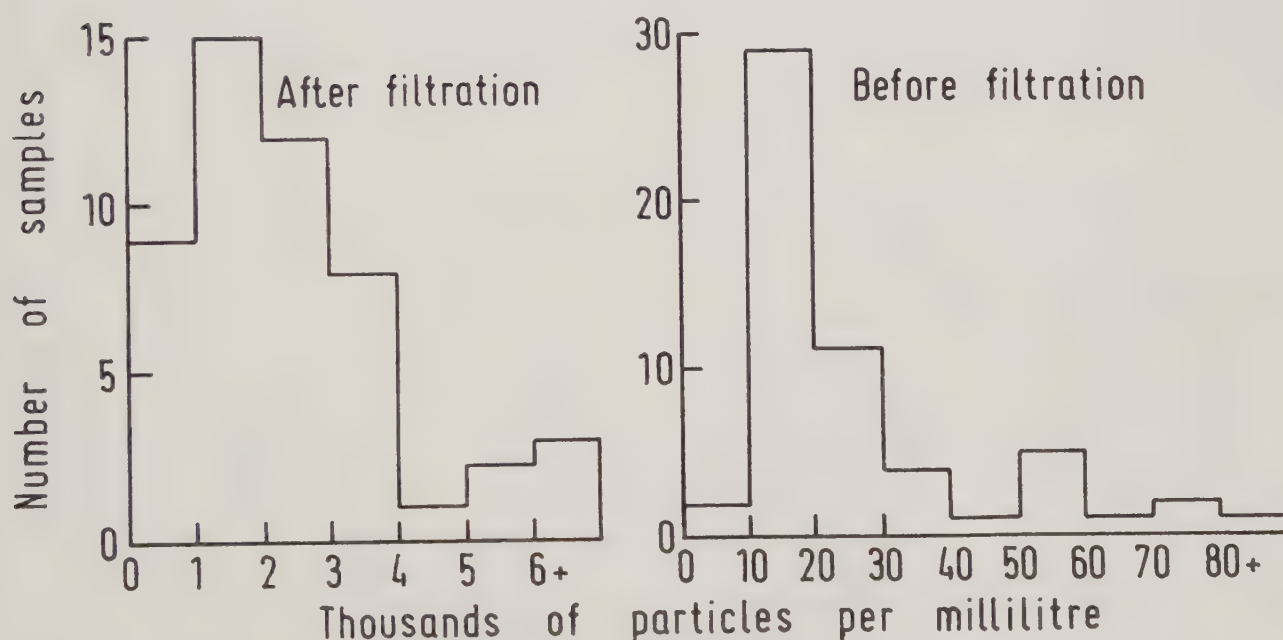


Figure 1 The number of particles larger than $17\mu^3$ (equivalent to a sphere 3.2μ in diameter) in 50 samples of sea water examined before and after filtration. Estimates made with a Coulter counter.

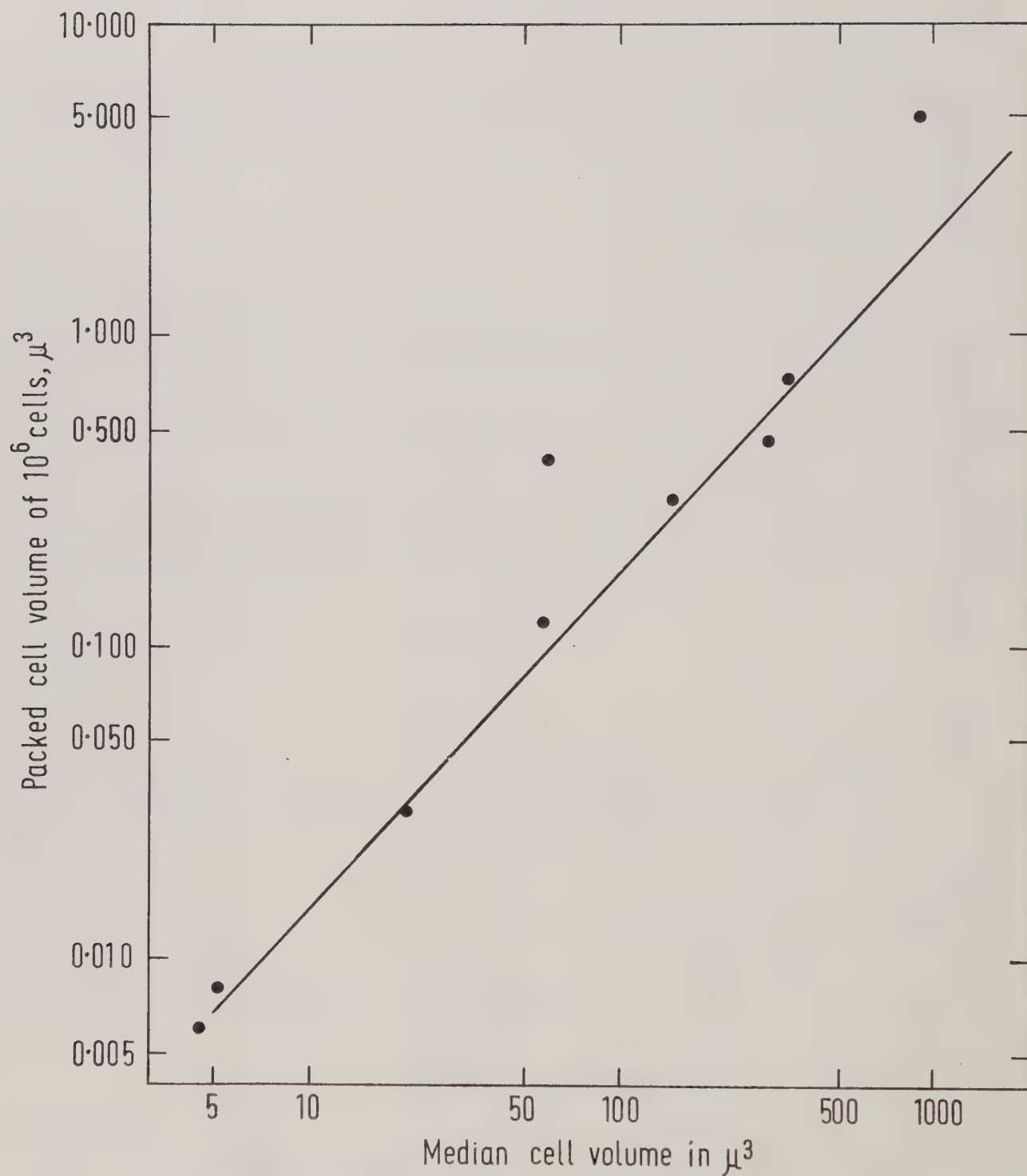


Figure 2 The relation between the packed cell volume and the median cell volume of a number of algal species, determined with a Coulter counter (see Table 2).

ALGAE

The species of algae and the origin of the strains used are given in Table 2; the classification is that given by Parke and Dixon (1964). Representative genera from the major groups were obtained, and the criteria of selection were size of cell and the readiness with which thick cultures could be obtained in our standard medium. As far as possible, described species have been used.

The same medium (Table 3) was used for all species throughout the work. This is more complex than required by some species but its use ensured that feeding different algal species would result in a similar degree of enrichment, on an inorganic basis and possibly of vitamins as well, of the experimental medium. An exception was made with the diatoms *Thalassiosira* and *Skeletonema*, where the culture was further enriched with 40 mg of sodium metasilicate per litre.

Most of the species were cultured in the semi-continuous apparatus described in detail elsewhere (Walne 1966), but it was found that some species did not thrive in these conditions and grew better in stagnant culture. They were: *Brachiomonas*, *Dicrateria*, *Micromonas*, *Nannochloris*, *Pyramimonas*, *Thalassiosira* and *Chaetoceros*.

The algae were fed to the bivalves on the basis of cell numbers, because this has been found to be the quickest way of estimating the density of an algal culture, but it does not take any account of the substantial differences in cell volume and cell content between the different species. From time to time an estimate was made of the cell volume in one of two ways and the average results are given in Table 2. The packed cell volume was found by centrifuging 40 ml samples of culture, resuspending the cells in 1 ml of sea water and again centrifuging in a haematocrit tube. The volume is expressed as the volume occupied (in μl) by 10^6 cells. The other method used the Coulter counter (see Sheldon and Parsons, 1966, for discussion of its application to algal cultures) and this measured directly the volume of particles suspended in an electrolyte. For the present work sufficient algal culture was diluted to 250 ml with filtered sea water to give a suspension of about 20 000 cells per ml. The counter was calibrated with pollen grains and the cell sizes are expressed in μ^3 . It was found that the frequencies of various cell volumes in a culture were approximately normally distributed, and a useful measure of the cell size of a particular species was given by the median volume. This was found to be very constant for a given species grown in standard conditions. It was also closely related to the packed cell volume (Figure 2) which indicates that both methods of measurement are a satisfactory index of the cell size.

Table 3 Stock enrichment solutions for the culture of *Isochrysis galbana* and other algae

1. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	2.60 g	2. The trace metal solution has the following composition	
$\text{MCnI}_2 \cdot 4\text{H}_2\text{O}$	0.72 g	ZnCl_2	2.1 g
H_3BO_3	67.20 g	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	2.0 g
E.D.T.A. (Na salt)	90.00 g	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	0.9 g
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	40.00 g	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	2.0 g
NaNO_3	200.00 g	Distilled water	to 100 ml
Trace metal solution	2.00 ml	It is necessary to acidify this solution with HCl to obtain a clear liquid	
Distilled water	to 2 litres	3. Vitamin: stock solution	
1 ml is added to each litre of sea water		B_{12}	10 mg
		B_1 (Thiamine)	200 mg
		Distilled water	to 200 ml
		10 ml are added to each 100 litres of sea water	

TREATMENT OF EXPERIMENTAL VESSELS

All the experiments were made in either 1 litre hard glass beakers or polythene vessels containing 8, 16, 25 or 50 litres of sea water. The volume of water used depended on the size and number of animals in an experiment, and it was adjusted so that the filtering activity of the animals would have a negligible effect on the algal population between changes of water. In all the experiments the water was changed three times a week: on Monday, Wednesday and Friday. During the change the animals were removed, the vessel cleaned with hot water and detergent, well rinsed, and then refilled with freshly filtered sea water and the appropriate quantity of algal culture. Clams were cleaned by washing on a sieve under a jet of fresh water; plates with attached oysters were lightly brushed and rinsed in fresh water. Occasionally the animals were also rinsed in 3 p.p.m. of chlorine. The water was kept stirred by aeration from a single glass jet held near the bottom of the tank.

Most of the experiments were made in the temperature range 21–23°C, which occasionally increased to 20–24°C. The 1 litre beakers usually stood in water baths for temperature control and in this situation they received a rather dim natural light. The other vessels stood on shelves in a controlled temperature room where they only received some artificial light during the working day.

The glass plates bearing oyster spat usually lay flat on the bottom with the side bearing oysters facing upwards, although in some experiments the plates were suspended. Clams lay at random on the bottom.

EXPERIMENTAL PROCEDURE AND REPORTING THE RESULTS

The aim of the investigation was to make a broad survey of the food value of a number of species of algae. With each food it was hoped to obtain the maximum growth possible, and to compare that with the growth in a standard control. To accommodate this each test comprised a series of experiments in which samples of the same batch of animals were fed at a number (usually five) of different concentrations of the algae under examination. It was hoped that one or more of the concentrations used would support maximum growth. *Isochrysis* was normally used as the control food, but *Tetraselmis* was used in some experiments. The amount of growth obtained in the controls varied from time to time. Doubtless this was partly due to the variation in the growth characteristics of the different animals, but there is also some evidence that variations in the quality of the sea water were exerting an influence. This will be examined in more detail in a later paper. To guard against a test food being unsuitable at a specific time, perhaps because of a particular bacterial contaminant or because of water quality, most species were tested more than once and at widely separated times.

A number of tests, for examples see Figure 6 and App. Table 5, showed that filtered water did not support 'growth' as measured by increase in cell size. As this was markedly less than in the controls it was not thought necessary to include this test in each experimental series.

The results of all the experiments are shown as a series of App. tables, giving the amount of growth obtained in each cell density. In some cases figures giving the growth curve during the experimental period are also shown. In most experiments few deaths occurred and as nowhere were they related to the feeding conditions, these data are not included. Occasionally a defective batch of animals which either failed to grow, or had abnormally high mortality rates, was encountered, and the results from these have been discarded.

Haptophyceae

DICRATERIA INORNATA

This species is a yellow-brown flagellate with a cell volume about 37 per cent of that of *Isochrysis*. It was first used successfully as a food for oyster larvae by Bruce, Knight and Parke (1940) under the name of flagellate H. Stagnant cultures had to be used in the present work, since it does not grow well while being continually stirred.

Juvenile *Ostrea edulis* were grown on this food in two series of experiments for 26 and 22 days respectively. A range of 50 to 300 cells per μl was tested; the results obtained (App. Table 1) were very similar and the combined results (Figure 3A) show that the best growth was at about 125 cells per μl . The *Isochrysis* controls, which gave a little poorer growth in one series and a little better in another, suggest

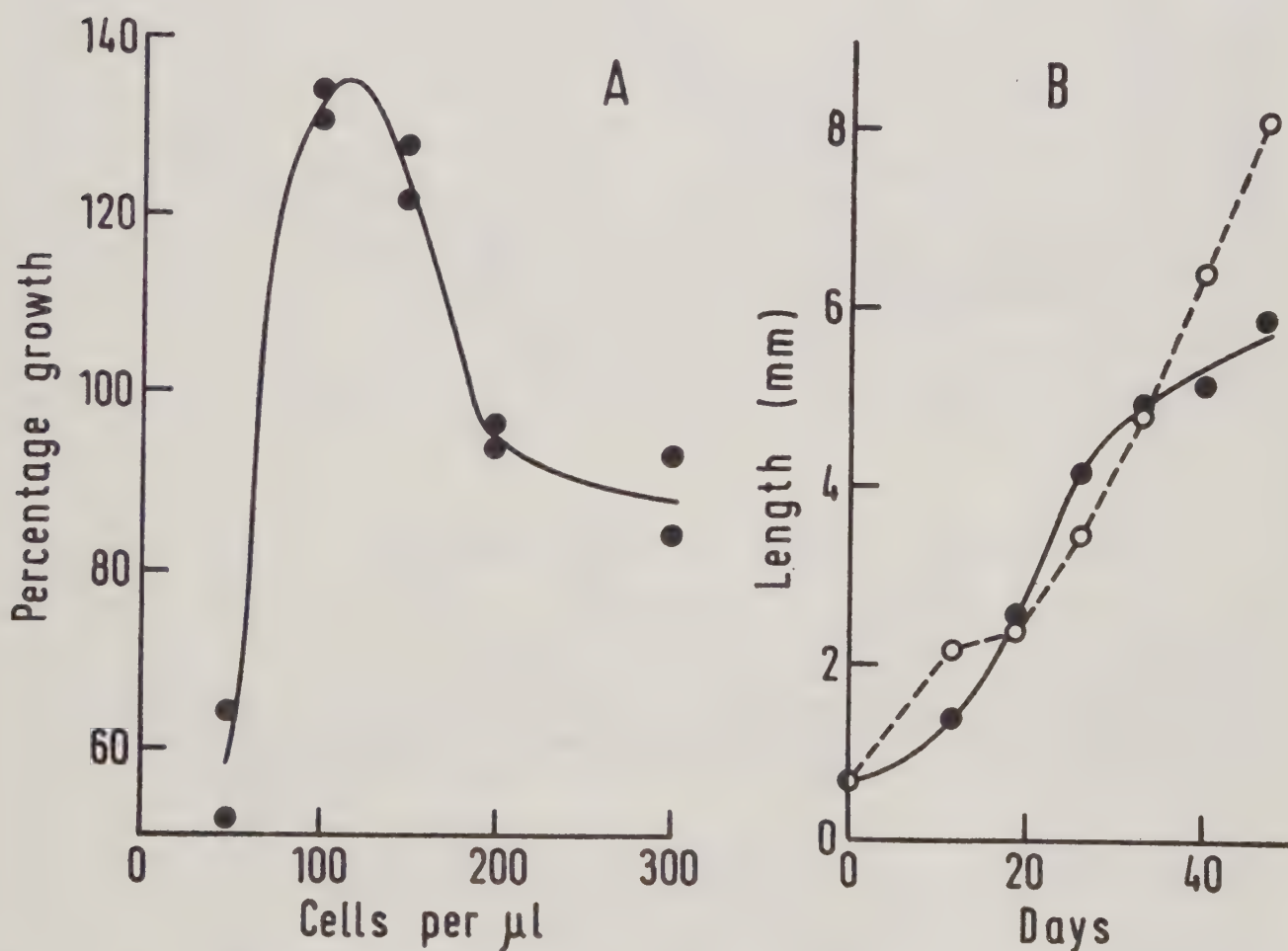


Figure 3 The growth of *Ostrea edulis* when fed on *Dicrateria inornata* in two experiments. A shows the growth at different cell densities in two experiments, expressed as a percentage of the mean, after 26 and 22 days respectively; B shows the growth of the *Isochrysis* control from one of the experiments (broken line) compared with the growth of larvae fed with 100 cells per μl of *Dicrateria* (continuous line).

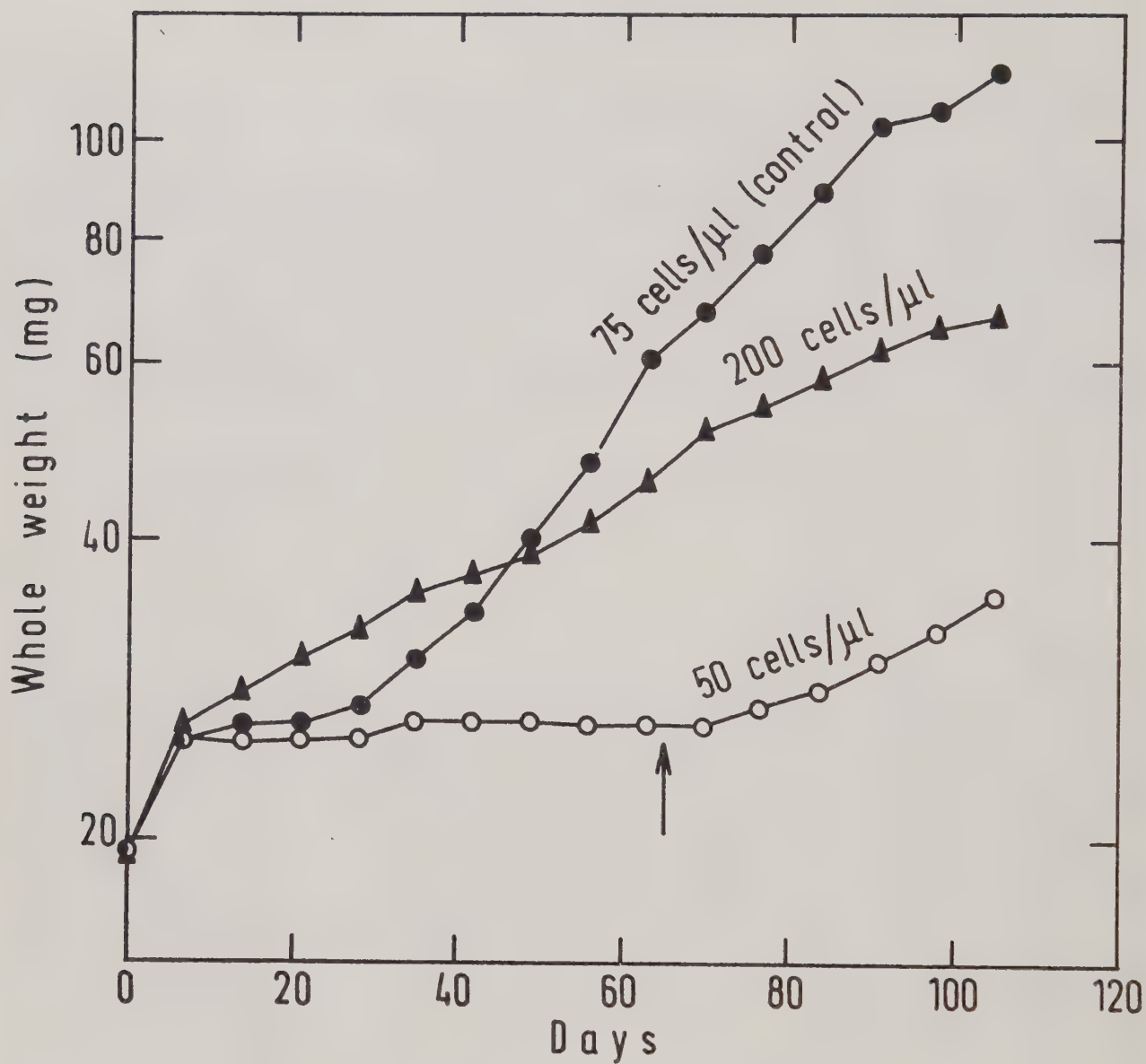


Figure 4 Growth of *Mercenaria mercenaria* when fed on *Dicrateria inornata*: solid circles, fed with 75 *Isochrysis* cells per μ l, triangles with 200 and open circles with 50 *Dicrateria* per μ l; the arrow indicates when the food of the latter was changed to 75 *Isochrysis* per μ l.

that the food value of the two species is similar. The observations were extended in the first series, where the controls and those fed at 100 cells of *Dicrateria* per μl were continued for 47 days (Figure 3B), by which time those fed on *Isochrysis* had a marked advantage. The following results show how in another experiment with *O. edulis* in February growth was little different over a period of 21 days when fed on *Dicrateria* in filtered and unfiltered water:

Concentration of <i>Dicrateria</i>	Mean length of <i>O. edulis</i> (mm)	
	Filtered water	Unfiltered water
100 cells per μl	6.0	6.0
50 cells per μl	4.5	6.1

Three series of experiments were made with *Mercenaria mercenaria* (App. Table 2) using animals with initial mean lengths of 0.6, 2.1 and 4.3 mm. The smallest animals, grown for 21 days in 140 ml test tubes, showed an increasing response at concentrations of between 100 and 300 cells per μl , with the best growth similar to that in the *Isochrysis* control. The 2 mm spat were tested over a lower range of food concentration and the growth rate at 100 cells per μl was less than in the control. In the third series, with 4 mm animals, the growth in 63 days was similar over the range 125–200 cells per μl and a little less than in the control. In this series three of the experiments were continued for a total of 112 days (Figure 4), and although *Dicrateria* at 200 cells per μl supported a constant growth rate it was not as high as in the control; 50 cells of *Dicrateria* per μl supported no growth at all, but when these animals were transferred to *Isochrysis* after 65 days growth soon restarted. The numbers of survivors out of 20 animals were 9 in the control and 13 and 16 in those fed on 50 and 200 cells per μl of *Dicrateria* respectively.

Dicrateria proved to be a good food for both species but considerable densities were required: 125 per μl for *O. edulis* and perhaps as much as 200 per μl for clams. We have found it to be a difficult species to rear because of marked seasonal fluctuations. For large-scale use a study of its cultural requirements would be required.

ISOCHRYISIS GALBANA

This species is employed as the standard food for bivalve larvae at Conway (Walne 1966) and its use has therefore received a good deal of attention (Walne 1965). The initial experiments demonstrated that it was a reasonable food for juveniles and it was then adopted as the standard food organism, against which other foods were tested. For this reason not only were a number of experiments made testing various densities of the food, but there are also numerous observations on the growth obtained when animals were fed at the same cell concentration but at different times of the year. The latter data will be examined in more detail in a further paper.

I. galbana is a yellow-brown flagellate which was first found to be an excellent food for oyster larvae by Bruce *et al.* (1940, as flagellate I). The strain used in that work still persists in culture and with another isolated from the River Crouch by Butcher has been used in the present study.

The results of six experiments in which various amounts were fed to *Ostrea edulis* are shown in App. Table 3. A convenient method of comparing the experiments is to plot the size of spat in one food concentration against the size of the same in another concentration. This is done in Figure 5 for the following pairs of cell concentrations: 5 and 10, 10 and 25, 25 and 50, 50 and 75 per μl . Little difference in growth was found between those fed at 5 or 10 cells per μl , but a marked improvement was found when the food was increased from 10 to 25 cells per μl . A further increase to 50 cells per μl did not, on balance, bring about enhanced growth. Food concentrations of 75 cells per μl and higher were unfavourable. Until

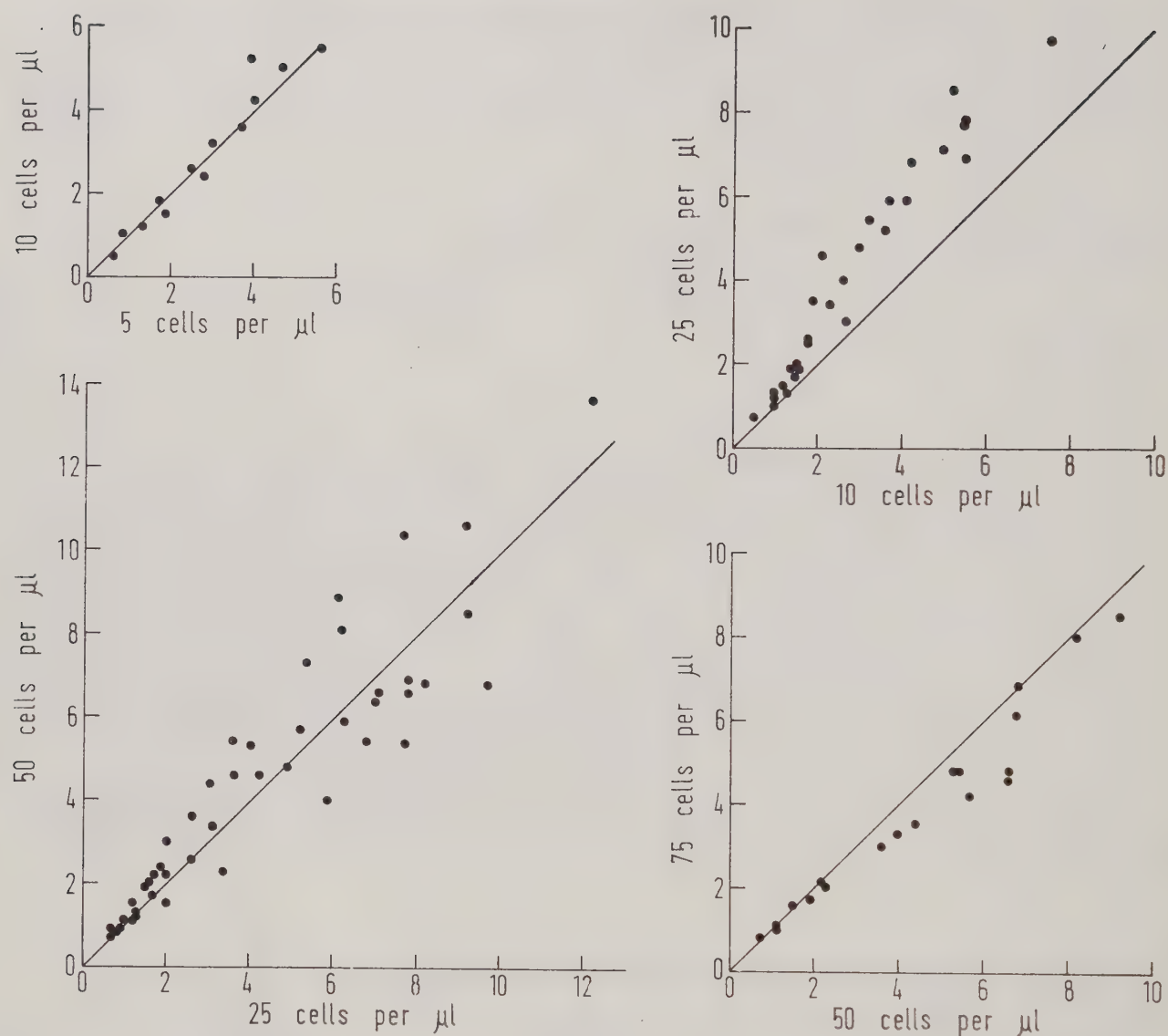


Figure 5 The growth of *Ostrea edulis* when fed on various concentrations of *Isochrysis galbana*. Each point represents the mean size (in mm) of one population of juveniles fed at one concentration of *Isochrysis* compared with a similar population fed at another concentration.

Table 4 The mean size (mm) of juvenile *O. edulis* after growing for 14 days. Initial size 0.70 mm

	Unfiltered sea water		Filtered sea water	
	Cells per μ l		Cells per μ l	
	25	50	25	50
	—	—	—	—
Live <i>Isochrysis</i>	1.89	3.26	2.22	2.42
Dried <i>Isochrysis</i>	0.92	1.24	1.02	1.14

this picture became clear many control batches of spat were fed at 75 cells per μl , but the full data suggest that better results would have been obtained with these controls if they had been fed at 50 cells per μl .

Experiments with two other species of oysters, *Crassostrea gigas* and *Ostrea lutaria*, showed a similar pattern (Figure 6). Concentrations of 100 cells per μl and above were unfavourable, with good growth being obtained at 25 to 50 cells. It is noticeable that when 75 cells per μl were fed in unfiltered water excellent growth was obtained with both these species, and with *C. gigas* even higher densities of food supported good growth. This suggests that the poor response at the higher densities fed in filtered water was due not to the amount of suspended material in the water, but to some imbalance in the food supply.

Isochrysis was also a good food for clams but the response to different concentrations of food was different from that of oysters. Animals with an initial size of 0.4 to 4.5 mm (App. Table 4) grew as well or better at 100 cells per μl , compared with lower densities, and showed little diminution of growth at 150 cells per μl .

In one experiment the food value of freeze-dried *Isochrysis* to *Ostrea edulis* was compared with live cells. A sample of culture grown in the standard manner was dried for us by Dr. R. H. Harris of the British Museum (Harris 1964). By shaking the dried cells well with sea water a reasonably good suspension was obtained, although it contained some clumps of 2–12 cells. The results obtained (Table 4) did not suggest that *Isochrysis* treated in this manner could be utilized as food.

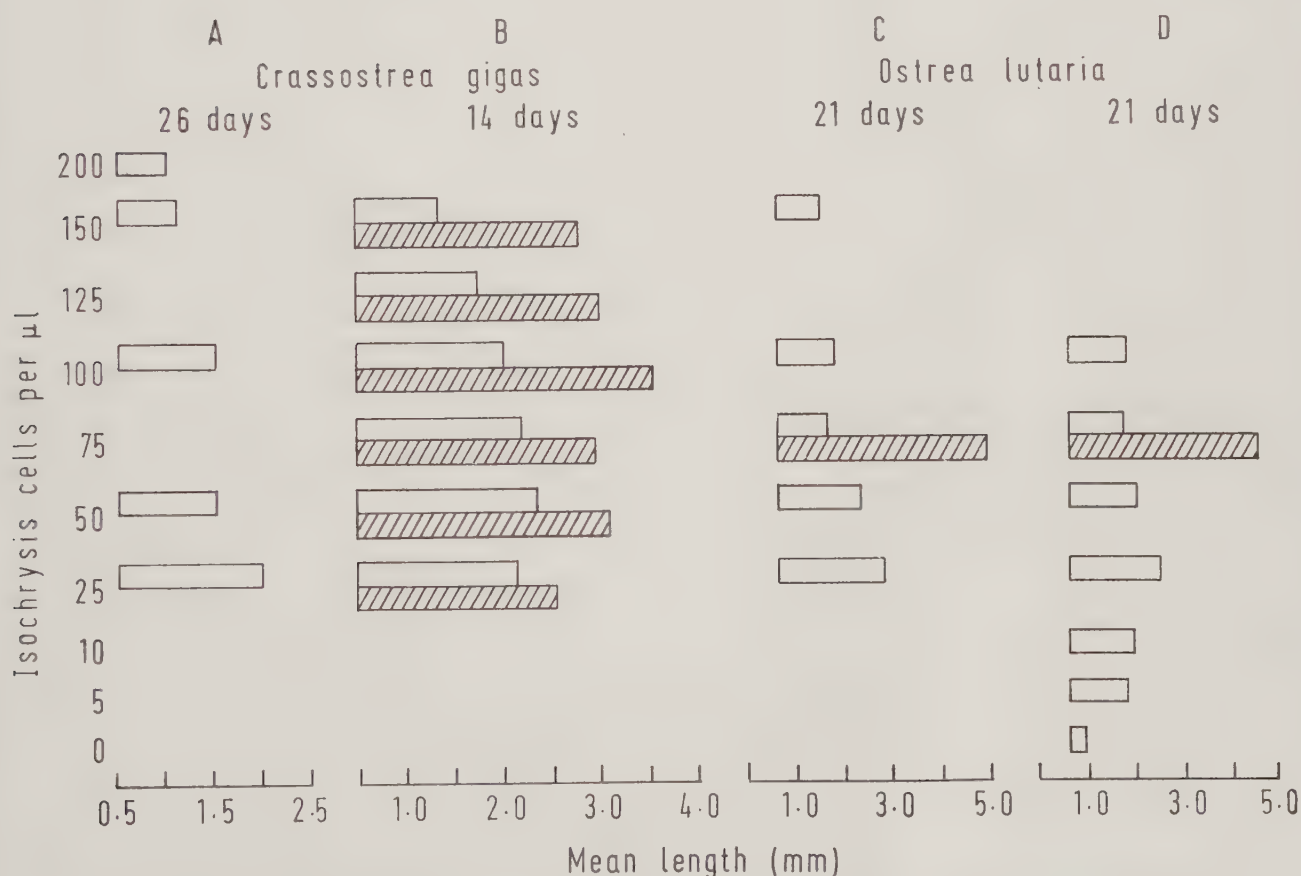


Figure 6 The growth of *Crassostrea gigas* and *Ostrea lutaria* when fed on *Isochrysis galbana*. The shaded blocks represent those experiments in which unfiltered water was used.

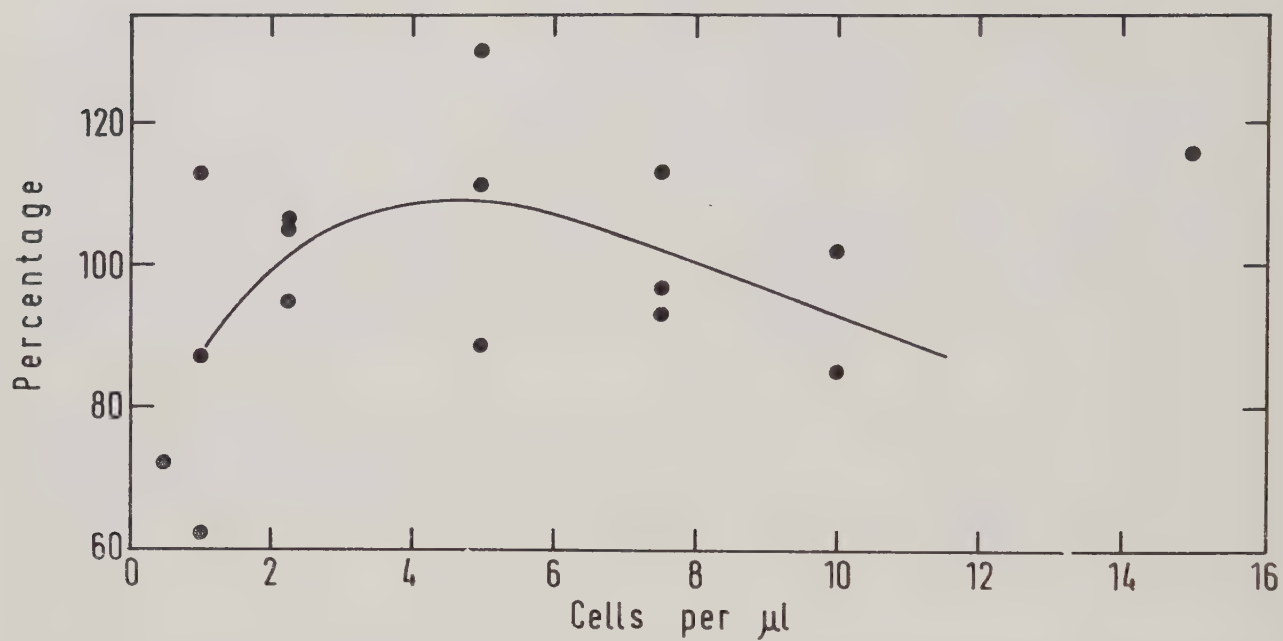


Figure 7 The growth of *Ostrea edulis* when fed on *Cricosphaera carterae*, shown as the mean size of *O. edulis* after 15 days expressed as a percentage of the mean size for the series when fed on *Cricosphaera*.

CRICOSPHAERA CARTERAE

This species, which was obtained as two separate strains (Plymouth 156 and 181), grew well in culture and had the largest cells of any of the flagellate foods tested. The two strains are very similar and probably belonged to the same species; strain 156 grew rather more vigorously in culture.

C. carterae was fed to three samples of *O. edulis* spat. Strain 156 and strain 181 were fed to the same sample (App. Tables 5, A and 6, B), and a further sample was fed with strain 156 (App. Table 5, C) and another with 181 (App. Table 6, A). The combined results shown in Figure 7 indicate similar rates of growth between 2.5 and 7.5 cells per μl . In each experiment the growth was better in the *Isochrysis* control. With *O. lutaria* (App. Table 5, B) growth, which was poor, was as good as in the *Isochrysis* control. Feeding with unfiltered sea water did not improve growth with either species of *Ostrea* (App. Table 5, B and C). The same batch of *O. edulis* spat tested with both strains indicates their similar food value.

Results similar to those obtained with oysters were obtained when both strains were fed to small clams (*M. mercenaria*). In two experiments some of the tests were continued for 70 and 42 days respectively (Figures 8, A, B) and these showed an approximately constant growth rate when the food was at either 5.0 or 7.5 cells per μl .

These results suggest that *Cricosphaera* is a reasonably useful food despite its size and tough cell wall, but it is less useful than *Isochrysis*.

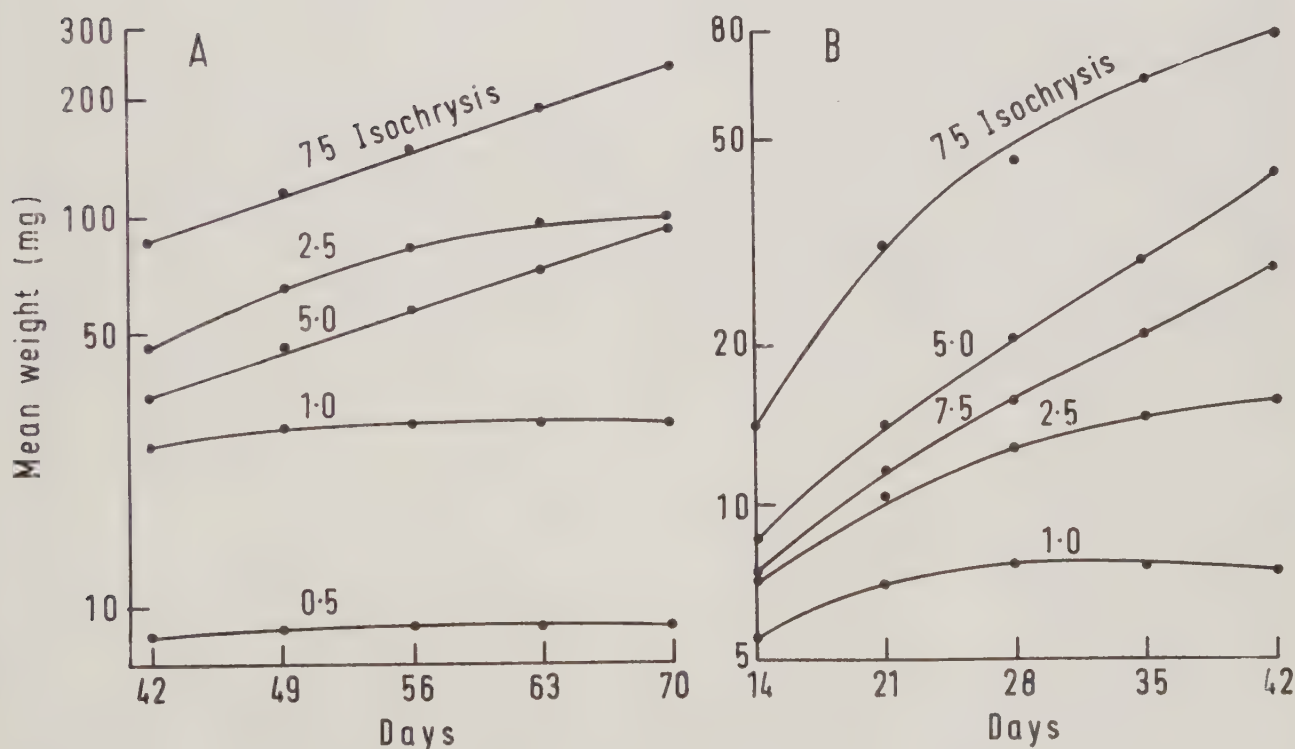


Figure 8 The growth of *Mercenaria mercenaria* when fed with various concentrations (cells per μl) of *Cricosphaera carterae*, compared with *Isochrysis* controls. A: Two experiments, duration 70 days; B: four experiments, duration 42 days.

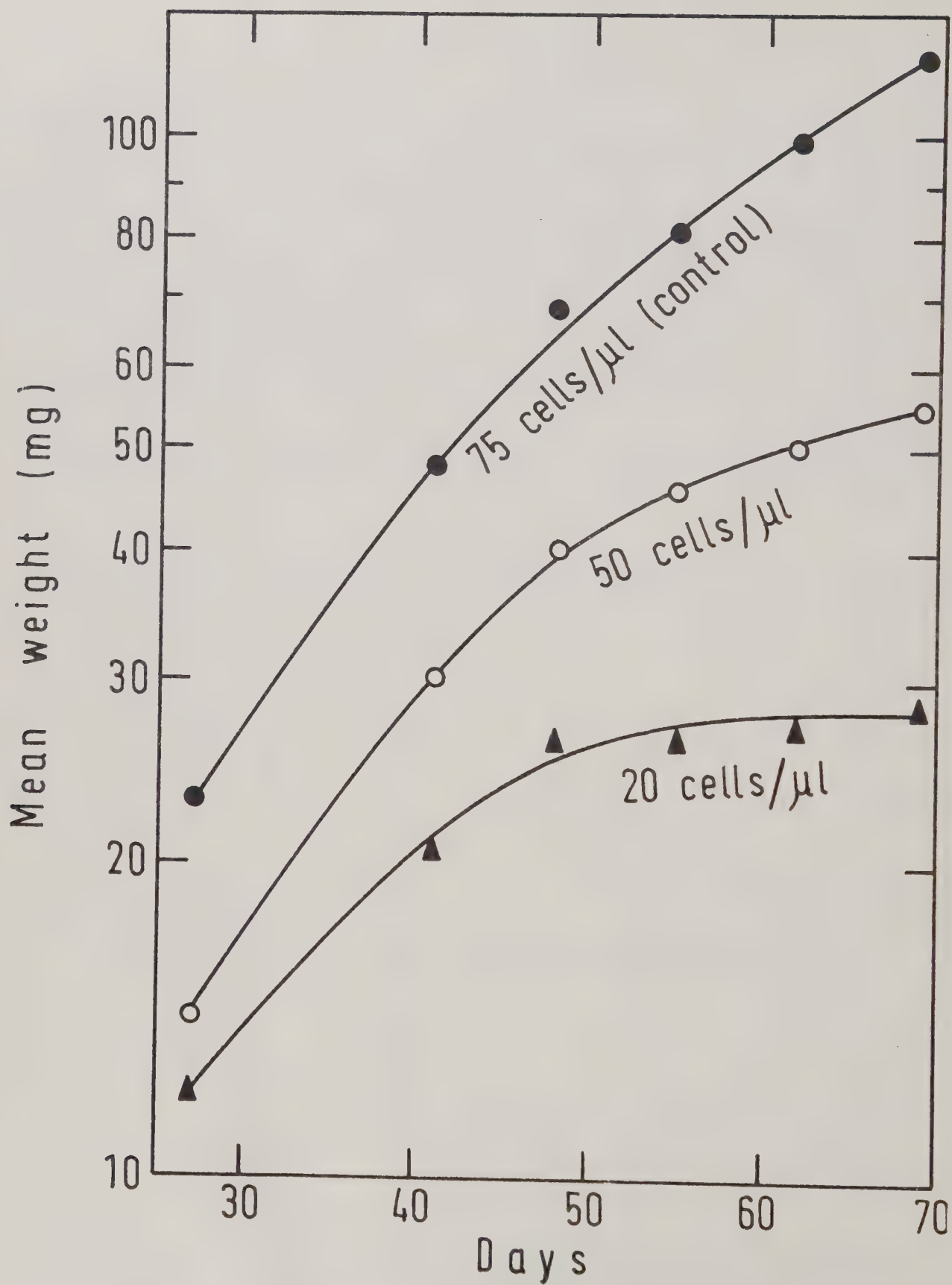


Figure 9 The growth of *Mercenaria mercenaria* when fed on 50 and 20 cells per μl of *Monochrysis lutherii*, compared with that of the *Isochrysis* control.

Chrysophyceae

MONOCHRYSIS LUTHERII

This species is a small yellow-brown flagellate which is widely used as a food for bivalve larvae and is the species preferred by some workers. The juveniles of three species of oysters – *O. edulis*, *O. lutaria* and *C. gigas* – have been fed on this food (App. Table 7). With *O. edulis* growth was as good as or better than in the *Isochrysis* control, but the relation with cell density was erratic. With *O. lutaria* the results were poor, with little growth occurring after 14 days; in this case the control was the exceptionally favourable combination of *Tetraselmis suecica* in unfiltered water. Good growth was obtained with *C. gigas*.

Three series of experiments were made with *Mercenaria mercenaria* and a steady increase in growth was obtained up to 100 cells per μl (App. Table 8), but in each case the controls fed with *Isochrysis* gave substantially better results. In some tests which were continued for 69 days the growth rate had become very slow at the end when fed with 50 cells per μl and had stopped in those feeding on 20 cells per μl (Figure 9). In general more useful results were obtained when this species was fed to oysters rather than clams.

PAVLOVA GYRANS

This species grows very well in continuous culture, but although suitable in size and closely related to good food species it has failed to support satisfactory growth in these experiments.

In an experiment with *Ostrea edulis* (App. Table 9, A) juveniles were fed at concentrations of 5 to 75 cells per μl . After 23 days those fed at the lower end of the range (5 to 25 cells per μl) showed very little growth, while those at the highest density, 75 per μl , were growing a little. Therefore those fed on the lowest cell densities (5 and 10 cells per μl) were finished, and those fed at 25 and 50 were increased to 100 and 150 cells per μl respectively and the experiment continued for another 14 days. In this period growth gradually came to a stop at both these concentrations, although it was continuing in the *Isochrysis* control. A similar experiment made with *Crassostrea gigas* gave negligible growth.

In one test (see Figure 14) clams were fed at 75 cells per μl in filtered and unfiltered water. Poor growth was obtained in filtered water but the animals in unfiltered water grew better than the control, indicating that some utilization was taking place. The result was, however, poor compared with several other foods. Moderate growth was obtained with *P. gyrans* in another series of observations (App. Table 9, C), but it was markedly poorer than in the control.

Prasinophyceae

MICROMONAS PUSILLA

This minute species is probably very common in the sea (Knight-Jones and Walne 1951) but because of its small size large numbers are required for an animal to obtain the same volumes of food as with larger species; one *Isochrysis* cell is, on a volume basis, equivalent to about 13 *Micromonas* cells.

Three experiments, covering the range of 50–2 000 cells per μ l, were made with *Ostrea edulis* (App. Table 10, A, B, C). The higher densities generally gave the best results, but although growth continued at a fairly constant rate for up to 36 days it was always markedly less than that obtained in the controls fed with *Isochrysis*. Feeding in the presence of unfiltered water (Figure 10) did not lead to an improved result.

In one experiment the control fed with *Isochrysis* was in duplicate; after 21 days one of the pair was moved into *Micromonas* at 100 cells per μ l. After 38 days the other plate was also moved to *Micromonas* and Figure 11 shows the course of the growth. There were 21 spat on the plate changed on the 38th day and 11, with a size range similar to the whole population, were removed and the average dry meat weight estimated. This was 0.89 mg and after 7 days' feeding on *Micromonas* the average meat weight was 0.81 mg. These results demonstrate that the larger juveniles, despite the better development of their gills, do not grow better than the newly-metamorphosed spat on this food.

Only a little growth occurred with *C. gigas* (App. Table 10, D, E) but there was some evidence that when *Micromonas* was fed in unfiltered water it did act as an additional food.

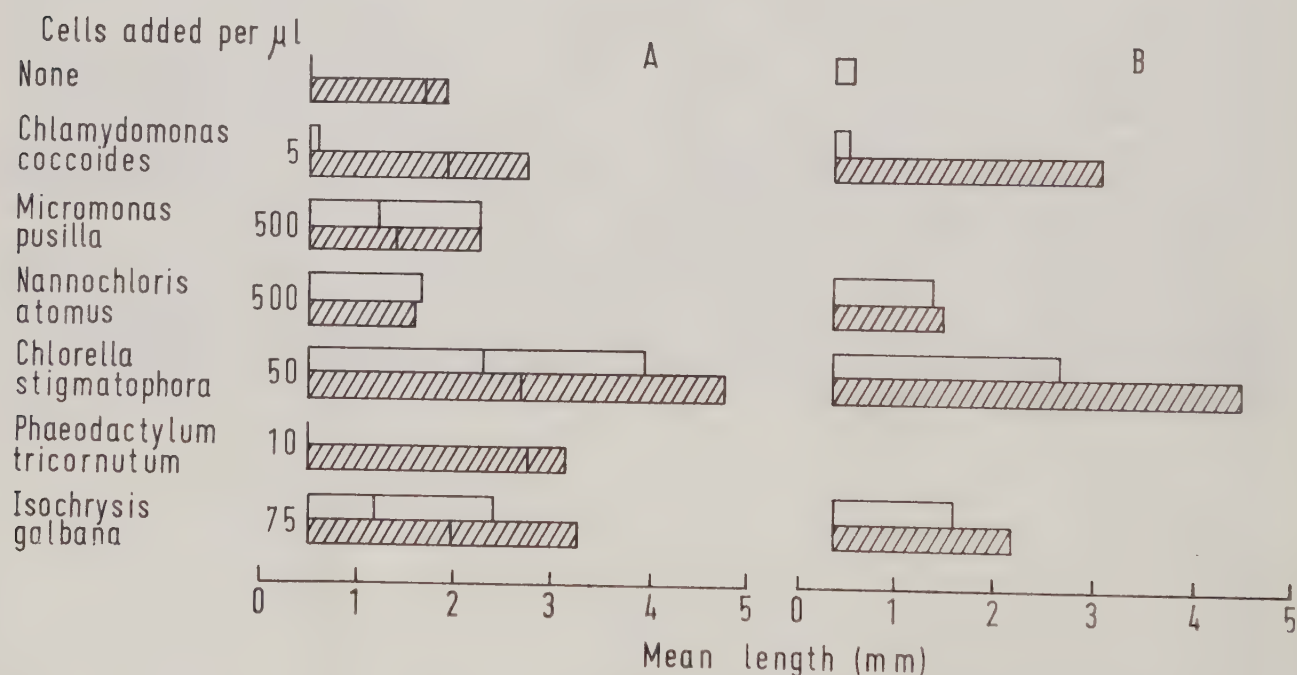


Figure 10 The growth of *Ostrea edulis* when fed on various algal species in filtered (plain blocks) or raw sea water (shaded blocks). In A the vertical lines represent the mean size after 15 and, in those which were continued, 21 days. The series of experiments represented in B were started 2 days after A with animals of different origin; the experiment was continued for 21 days except for *Nannochloris atomus*, which was finished after 17 days because the plate in raw sea water was damaged.

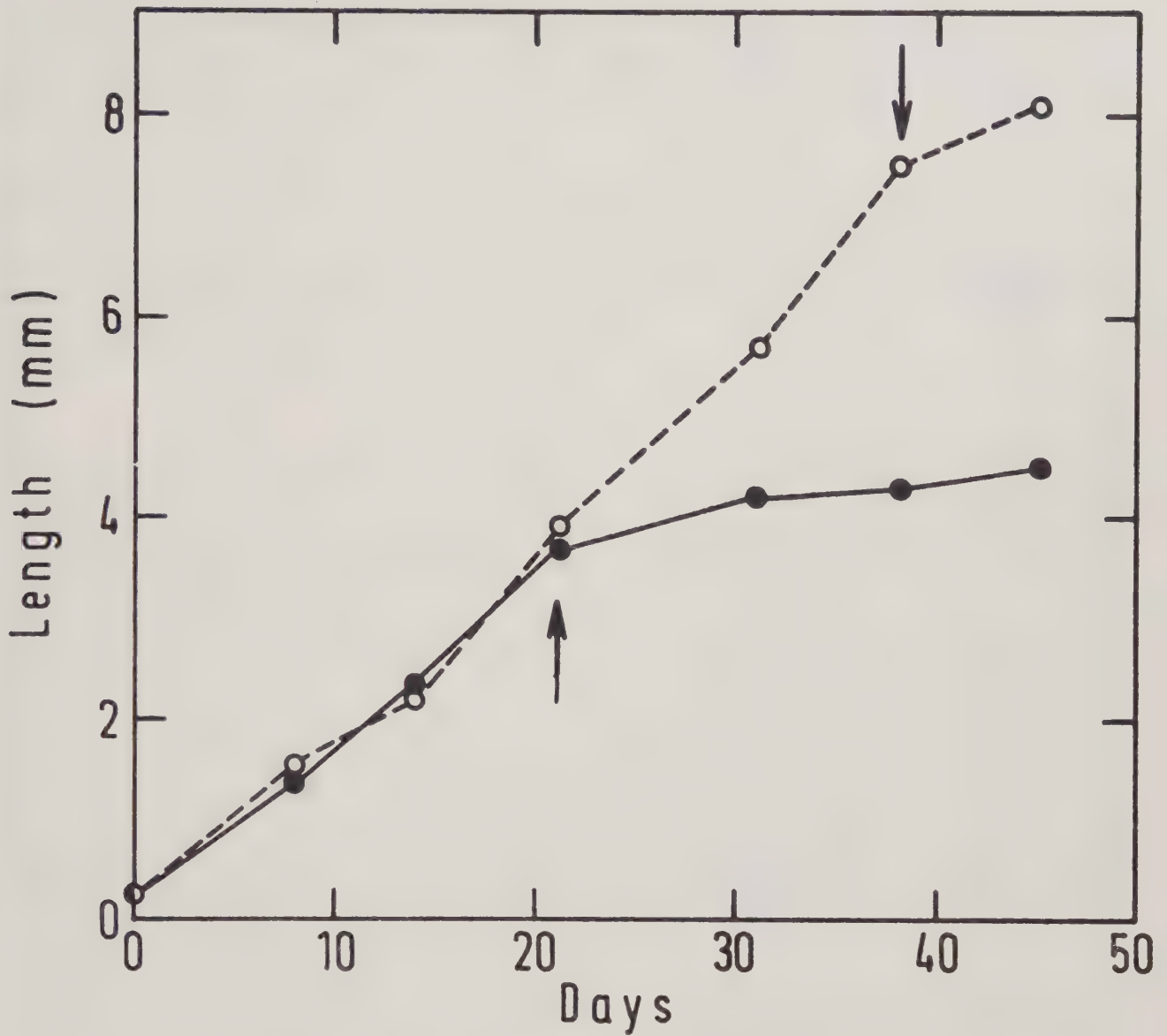


Figure 11 The growth of *Ostrea edulis* in an experiment in which duplicate controls were fed initially on *Isochrysis galbana* at 50 cells per μl and changed to *Micromonas pusilla* at 100 cells per μl , one after 21 days and the other after 38 days (arrowed).

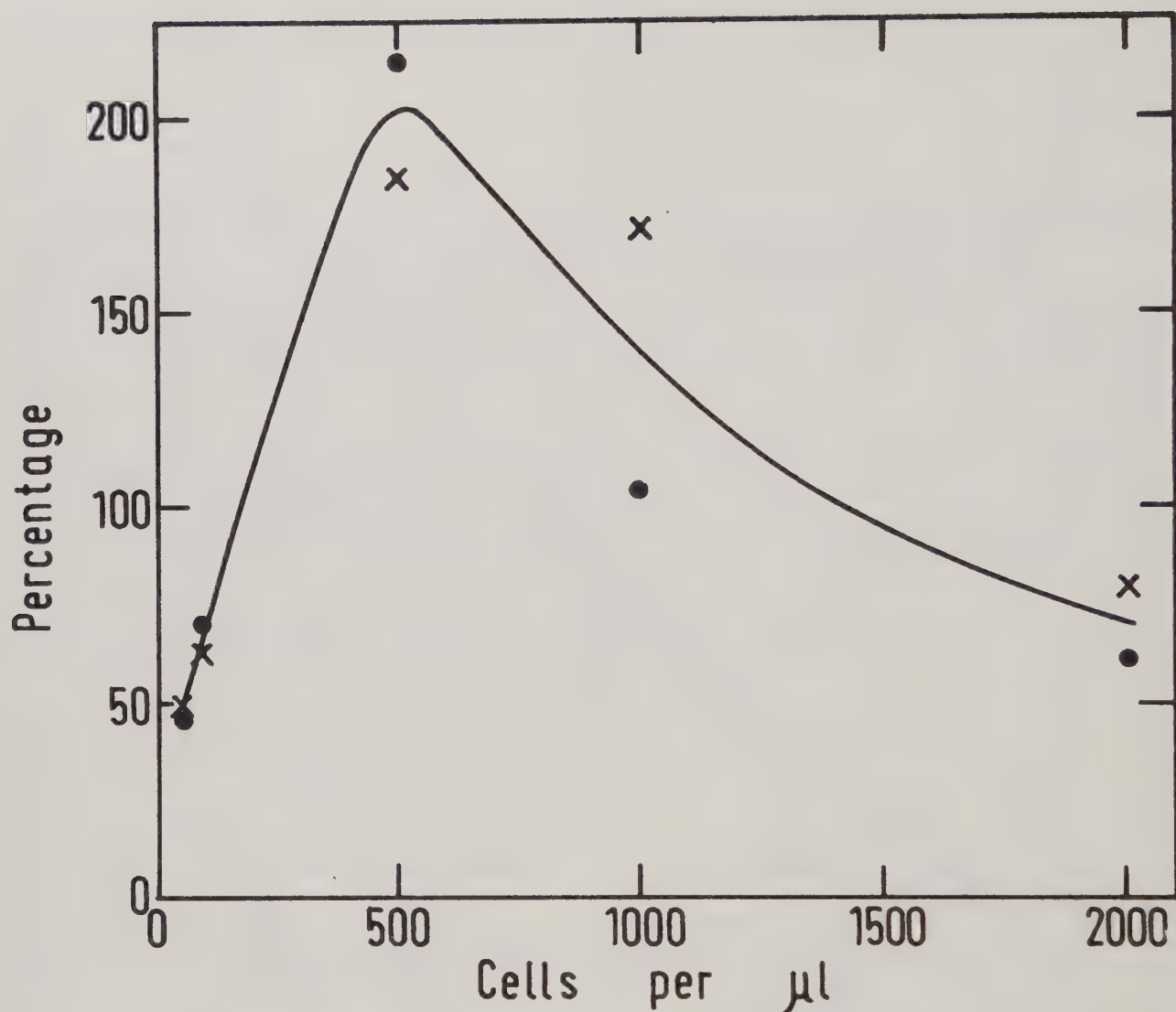


Figure 12 The growth of *Mercenaria mercenaria* when fed on *Micromonas pusilla* for 21 days, shown as the growth at different cell densities as a percentage of the mean; calculated from the final results of the experiments shown in Appendix Table 11, A (circles) and B (crosses).

In two experiments where clams were grown for 21 days on *Micromonas* fairly good growth was obtained in the region of 500 to 1 000 cells per μl (App. Table 11, A, B) although there was some evidence that the rate was declining (Figure 12). In both experiments in which *Micromonas* was fed in filtered and unfiltered water (Figures 13 and 14) very good results were obtained in unfiltered water.

MICROMONAS SQUAMATA

This is a larger species than *M. pusilla* and in one experiment where it was fed to *O. edulis* (App. Table 11, C) the growth obtained was better than that found for *M. pusilla*.

TETRASELMIS

During this work it became apparent that species of *Tetraselmis* were of outstanding value as food for juvenile bivalves. Four species, *T. suecica*, *T. inconspicua*, *T. chui* and *T. tetrahele*, grew well in large-scale culture and they were all tested. *T. suecica* was used as a control food in a number of experiments. As *Platymonas* this genus was tested on juvenile *O. edulis* by Cole (1937) and found to be a good food.

One characteristic of this genus is that when a culture is first added to sea water most of the cells cease to swim and apparently become attached to the bottom. After some hours many cells start swimming, and by the following day the numbers swimming in the water approximate to the number originally introduced.

T. suecica was fed at a range of concentrations to *O. edulis* in three series of experiments (App. Table 12). Growth was as good as or better than in the *Isochrysis* controls. The response to the various concentrations was uniform (Figure 15) and equally good results were obtained in the range 10 to 30 cells per μl . In two experiments *T. inconspicua* was used as food and single tests were made with *T. tetrahele* and *T. chui*; all gave good results (App. Table 13), although the maximum growth rate required about 15 cells per μl . A comparison of three species in both filtered and unfiltered water showed no systematic difference between them (App. Table 14). A notable feature was the great stimulation given to growth when *T. suecica* was fed in unfiltered water. This will be dealt with in more detail in another paper; for the moment it is sufficient to say that a concentration of 2.5 cells per μl of *T. suecica* in unfiltered water produces outstanding growth in juvenile oysters.

T. suecica was fed to *C. gigas* in two series of experiments and good growth was obtained (App. Table 15, A, B); with *O. lutaria* growth was slow (App. Table 15, C) as was usual with this species on most foods.

T. suecica was fed to clams at a range of concentrations (App. Table 16). Growth was at least as good as in the *Isochrysis* controls, and at its maximum rate at about 15 cells per μl . In a further series of experiments a range of six concentrations of each of three species of *Tetraselmis* was fed to samples of the same batch of clams. The results after 21 days show that the growth obtained was rather similar with each food, with an optimum cell density of about 20 cells per μl (App. Table 17). The *Isochrysis* control, which was in duplicate, did badly, due, it is thought, to a defect in the culture, since, when at the end of the experiment the control animals were transferred to *Tetraselmis*, the growth rate quickly improved.

Tetraselmis is an outstanding food for juvenile bivalves, and since it is very hardy in culture it has considerable potential for use in large-scale rearing.

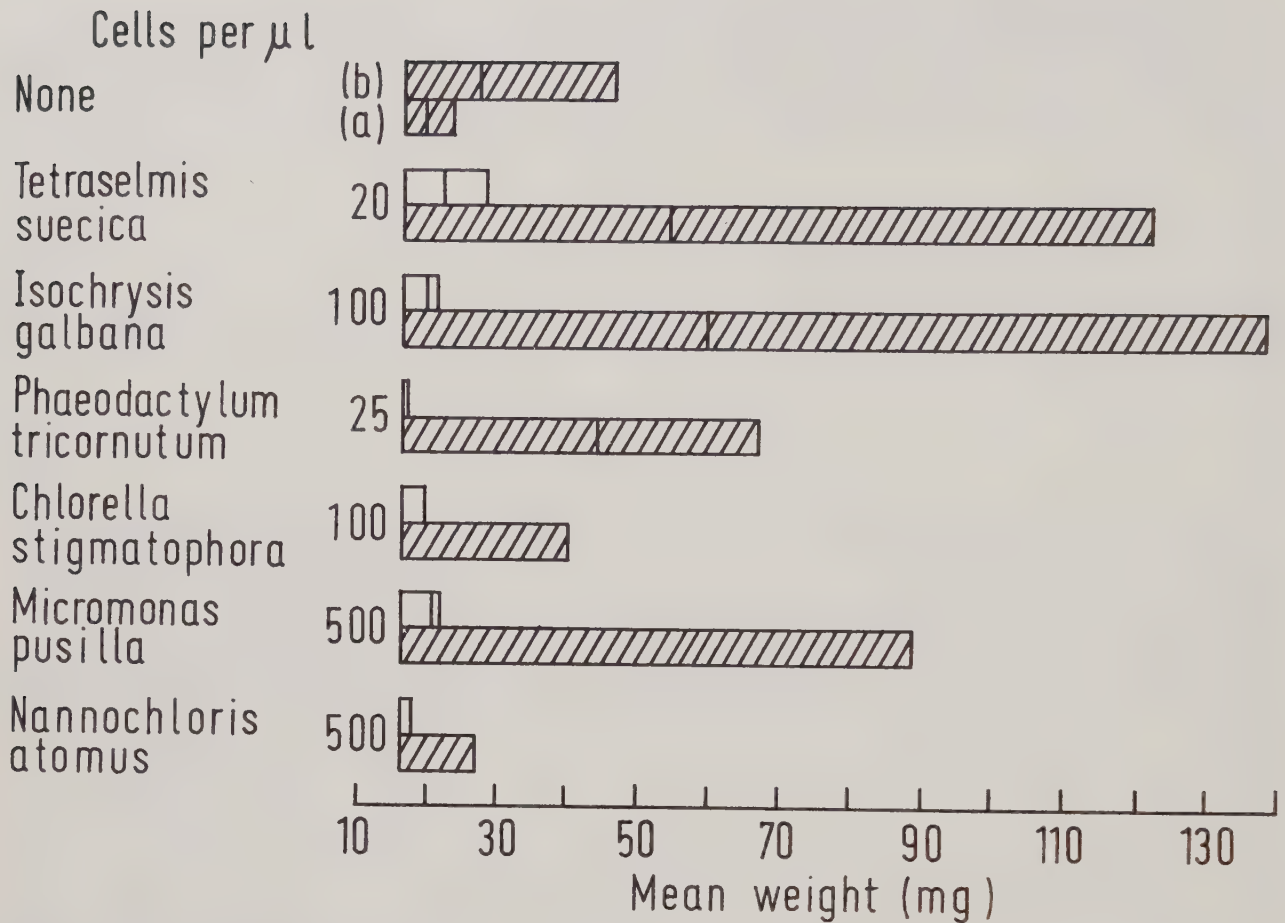


Figure 13 The growth of *Mercenaria mercenaria* when fed on various algal species in filtered (plain blocks) or raw sea water (shaded blocks). The experiment lasted for 48 days except for *Chlorella* and *Nannochloris*, which were finished after 28 days. The mean weight in all foods after 28 days is shown by vertical lines. In the experiments which were not enriched, the raw sea water for (a) was the laboratory supply used with the other foods, while (b) was collected in a bucket from the outside reservoir.

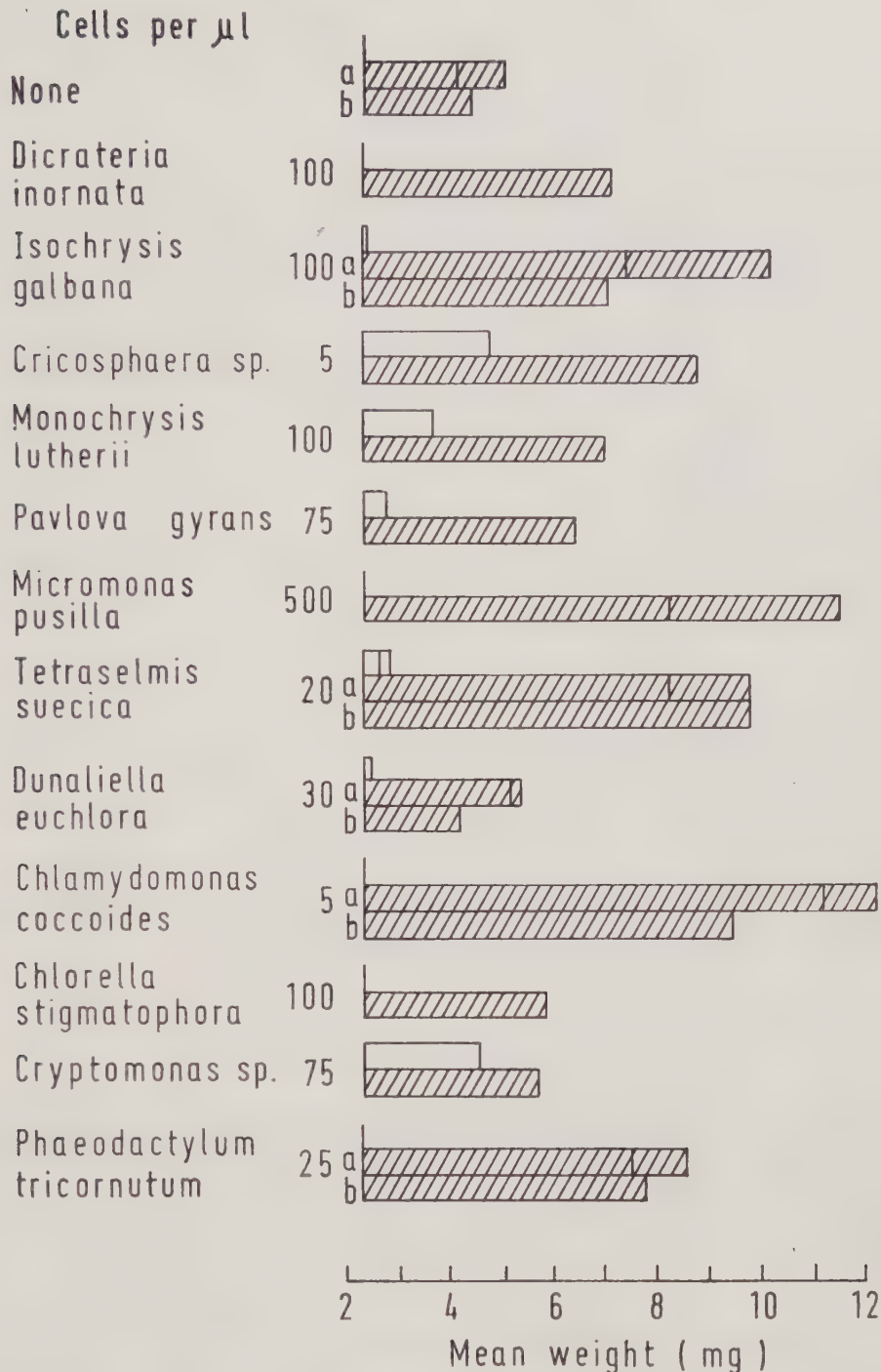


Figure 14 The growth of *Mercenaria mercenaria* when fed on various algal species in filtered (plain blocks) or raw sea water (shaded blocks): (a) indicates that the water was obtained from the laboratory supply and (b) that it was collected in a bucket from the outside reservoir. The vertical lines show the size after 21 days, when a number of experiments were finished, and 30 days. The experiments which were fed on *Chlorella* were finished after 14 days.

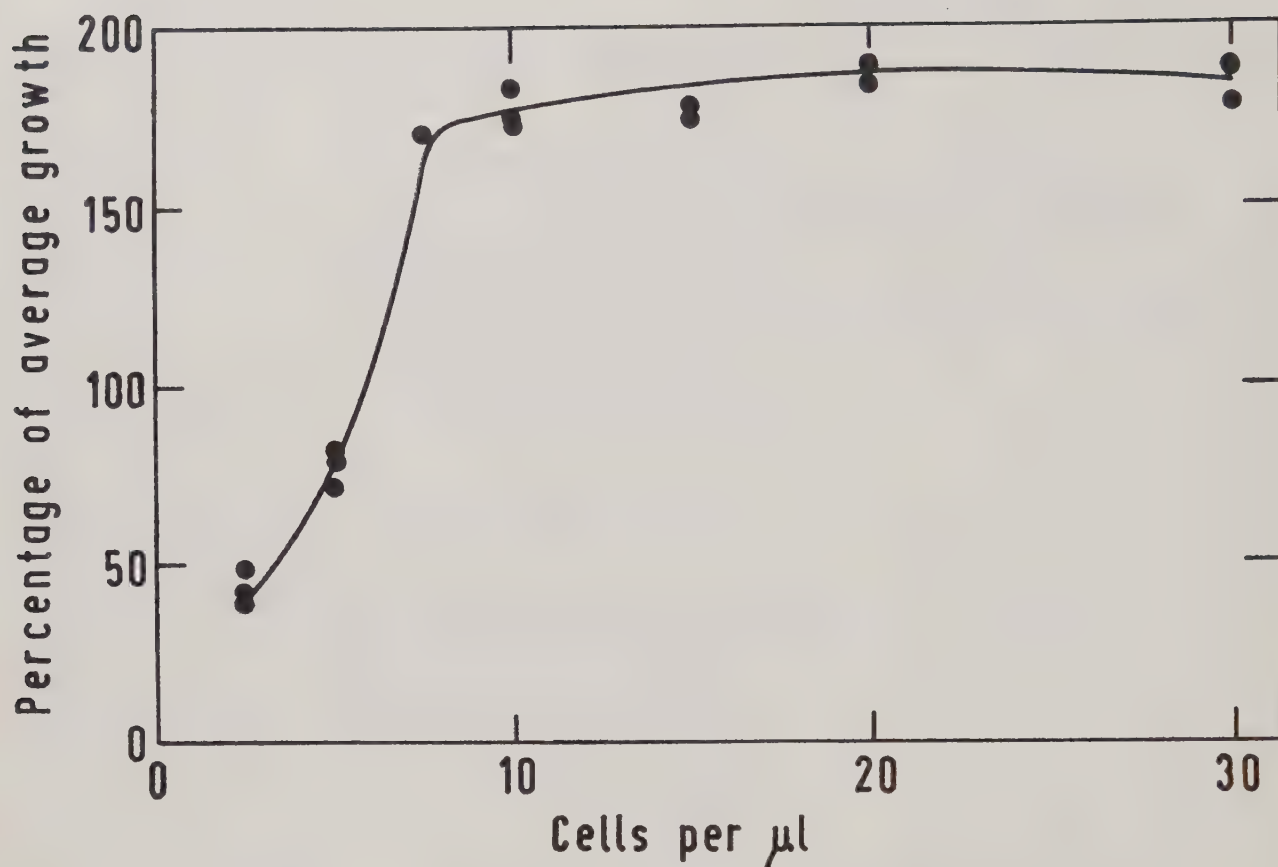


Figure 15 The growth of *Ostrea edulis* when fed on *Tetraselmis suecica*, shown as the growth at different cell densities expressed as a percentage of the mean.

PYRAMIMONAS GROSSII

This species was successfully used as a food organism for oyster larvae by Cole (1937) and by Bruce *et al.* (1940) as flagellate H, but this strain was lost in 1942 and the writer has found that the more recent isolations do not grow readily into thick cultures. For this reason it has only been possible to carry out one series of experiments, in which clams measuring 1.2 mm were fed for 14 days. The mean lengths at the end were as follows:

Cells per μl	100 <i>P. grossii</i>	75 <i>P. grossii</i>	50 <i>P. grossii</i>	25 <i>P. grossii</i>	75 <i>Isochrysis</i> (control)
Length (mm)	1.55	1.52	1.63	1.37	1.59

Apparently it was a reasonably nutritious food, but further examination of its cultural requirements are needed before more tests can be made.

Chlorophyceae

DUNALIELLA

Two species of *Dunaliella* were used in this work, *D. tertiolecta* and *D. euchlora*. The first was originally isolated by Mrs. Føyn from Oslo Fjord and subsequently maintained at Plymouth. It has been very widely used in the laboratory under the name '*Chlamydomonas* III' (Harvey 1947). Experiments with oyster larvae gave variable results (Walne 1963). The second species, *D. euchlora*, has given moderate results with the larvae of *Crassostrea virginica* and clams (Davis and Guillard 1958).

As both species of *Dunaliella* grow very well in culture, a careful assessment was made of their food value. In two series of experiments with *D. tertiolecta* and in one series with *D. euchlora* (App. Table 18) the final size of oyster spat fed with *Dunaliella* was very much less than those fed on *Isochrysis*. The sensitivity of the growth of oyster spat to *Dunaliella* was demonstrated when one batch of spat was fed alternately on 10 cells per μl of *Dunaliella* and 75 cells per μl of *Isochrysis* with two periods on each food over a period of 79 days (Figure 16, A). The curve clearly shows how quickly growth slowed up or stopped when the food given was *Dunaliella*. This experiment – which is a continuation of one of the *D. tertiolecta* series – was also made at each of the concentrations 10, 20, 30, 40 and 50 cells per μl of *Dunaliella*. The same step-like pattern of growth was obtained in all of them but differences were found in the mean size

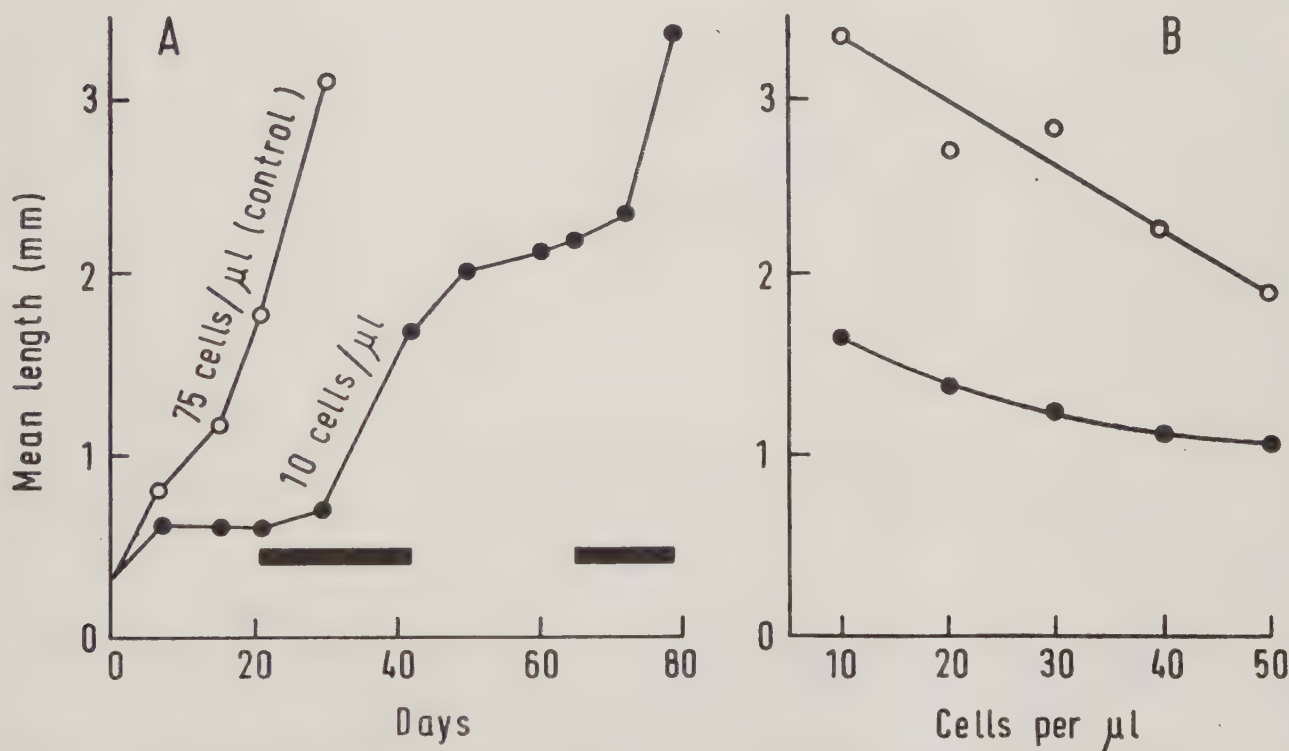


Figure 16 A: Growth of *Oostrea edulis* fed on 75 cells per μl of *Isochrysis galbana* (control) and on 10 cells per μl of *Dunaliella tertiolecta*; the latter were fed for two periods, shown by a horizontal bar, on *Isochrysis*, and B shows the mean size at the end of the first period (solid circles), and second period (open circles) of feeding on *Isochrysis*, related to the concentration of *Dunaliella* used in the alternate periods.

of the spat after periods of growth in *Isochrysis*. At the end of the first period of feeding in *Dunaliella*, there was little difference in the mean size of the five populations but after the subsequent period in *Isochrysis* the mean size of the spat was inversely related to the concentration of *Dunaliella* which had been fed to the spat in the previous period (Figure 16, B). The same phenomenon was seen in a more accentuated form at the end of the second period of feeding on *Isochrysis*. Apparently the higher concentrations of *Dunaliella* had so affected the spat that they were unable to grow so well when placed in a good food.

A similar experiment with alternately *Isochrysis* and *Dunaliella* was made with clams, only in this case *Isochrysis* was fed first (Figure 17). During the periods of feeding on *Dunaliella* there was again a sharp reduction in the growth rates, but in this series no relation was found between the mean size after feeding on *Isochrysis* and the concentration of *Dunaliella* as used previously. Very little growth was obtained with *D. euchlora* (Figure 14) in filtered or unfiltered water, compared with the controls.

None of these experiments suggest that *Dunaliella* is likely to be a useful food.

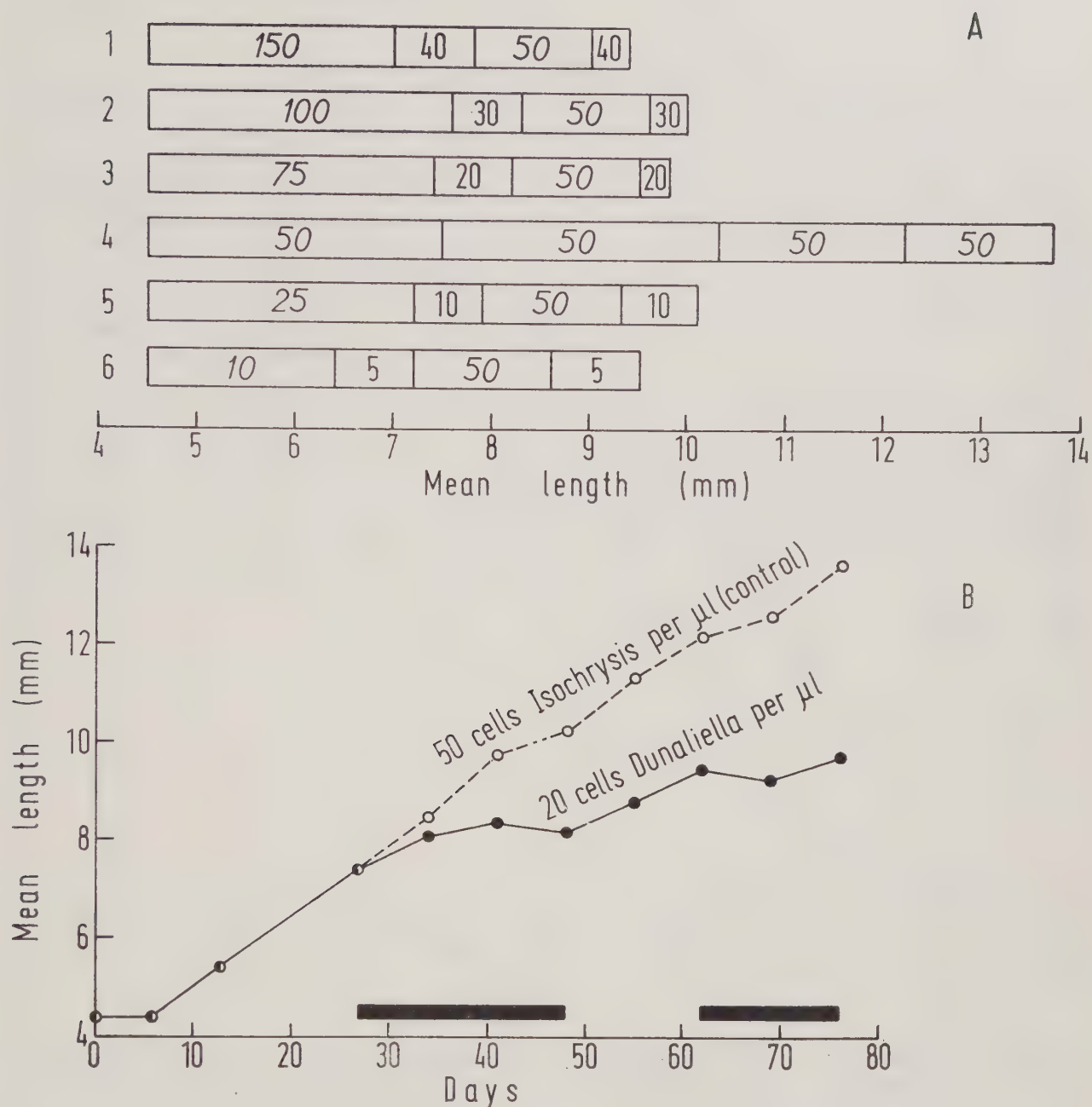


Figure 17 Growth of *Mercenaria mercenaria* when fed on *Dunaliella tertiolecta*. A: The growth when the animals were fed alternately on *Isochrysis* (italic numbers) and *Dunaliella* (upright numbers). The numbers show the cell density in μl . The four successive periods occupied 27, 21, 14 and 14 days respectively. B: The growth rate of the controls fed with 50 *Isochrysis* per μl and those fed with 20 cells of *Dunaliella* per μl during the periods indicated by horizontal bars.

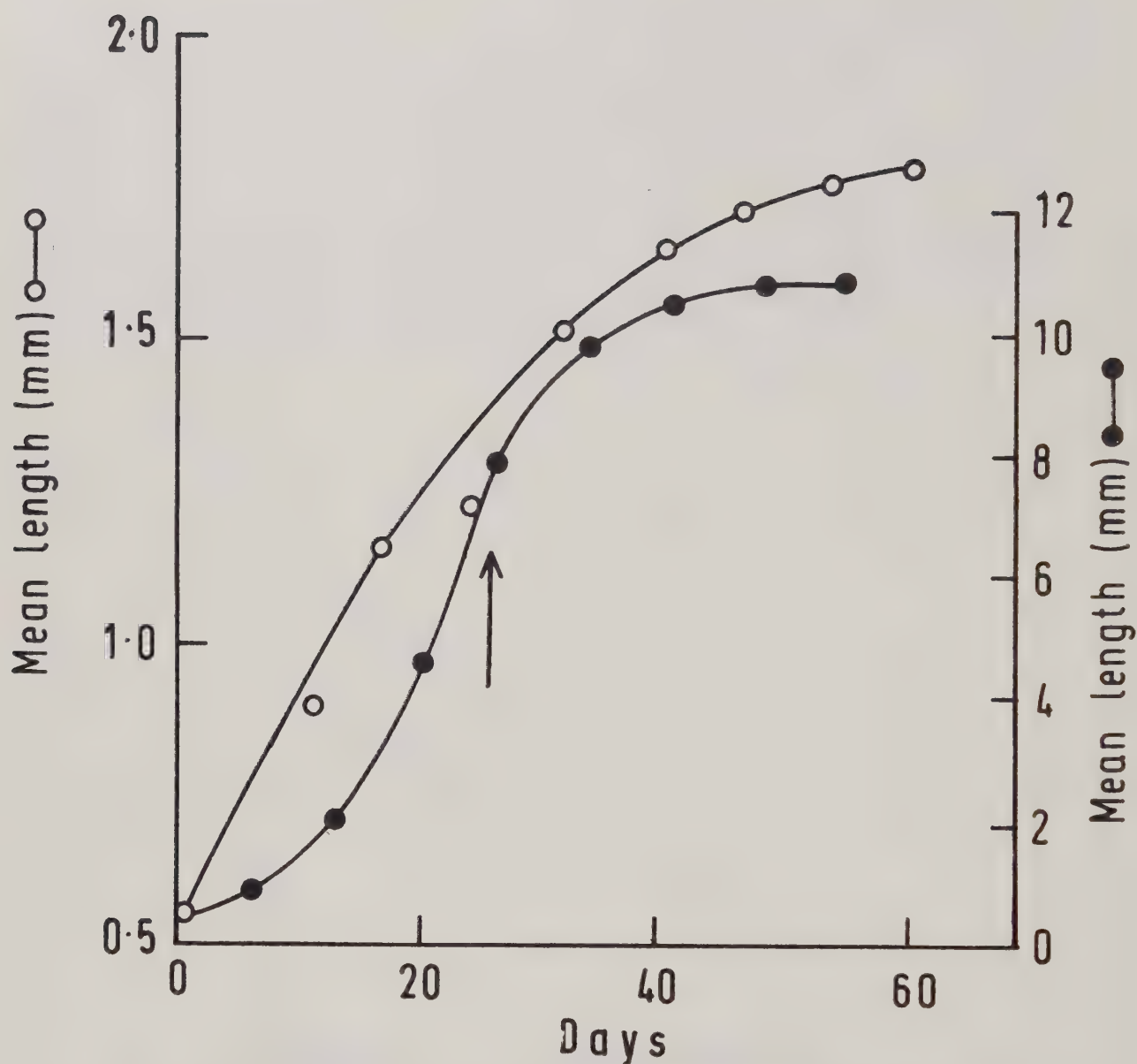


Figure 18 The growth of *Ostrea edulis* when fed on *Chlamydomonas coccoides*. The open circles show the growth rate of those spat which were fed on 5 cells per μl of *C. coccoides*, while the solid circles show the growth rate of those spat which were fed initially on 50 cells per μl of *I. galbana* and after 26 days (arrowed) changed to 5 cells per μl of *C. coccoides*.

CHLAMYDOMONAS COCCOIDES

Chlamydomonas is another genus of green flagellates which grows very well in culture. It is superficially similar to *Dunaliella*, but in *Chlamydomonas* the cell wall is a rigid structure; *Dunaliella* has no rigid cell wall.

Two series of experiments where this species was fed to *Ostrea edulis* covered the range 2.5 to 30 cells per μ l (App. Table 19, A, B); growth was poor in both series. In the first series most of the experiments were finished after 26 days, but those which had been fed with 5 cells of *C. coccoides* or 50 cells of *Isochrysis* per μ l were continued with 5 cells per μ l of *C. coccoides* for a further period (Figure 18). The growth of those which had been fed on the good food soon slowed down and stopped, and little further growth occurred in those fed on *Chlamydomonas*. This species supported little growth on its own, but there is some evidence that when it is fed in unfiltered water (Figure 10 and App. Table 19, C) some reasonable results can be obtained. The same applies to experiments with *C. gigas* (App. Table 19, E), but *O. lutaria* grew very little even in unfiltered water (App. Table 19, D).

Rather poor growth was recorded with clams fed on this species (App. Table 20), but the results of experiments where it was fed in unfiltered water (Figure 14 and App. Table 20, B) suggested that it was a useful food in these circumstances.

C. coccoides is an interesting species because, although it is a poor food on its own, it is apparently utilized when fed in a mixture. For clams it was comparable to *Tetraselmis* in those circumstances. Perhaps this is a species which is largely utilized by bivalves, but has some minor constituent missing, without which the bivalves are unable to grow. If this is so, then possibly the addition of small amounts of another food would allow the *Chlamydomonas* to be fully utilized.

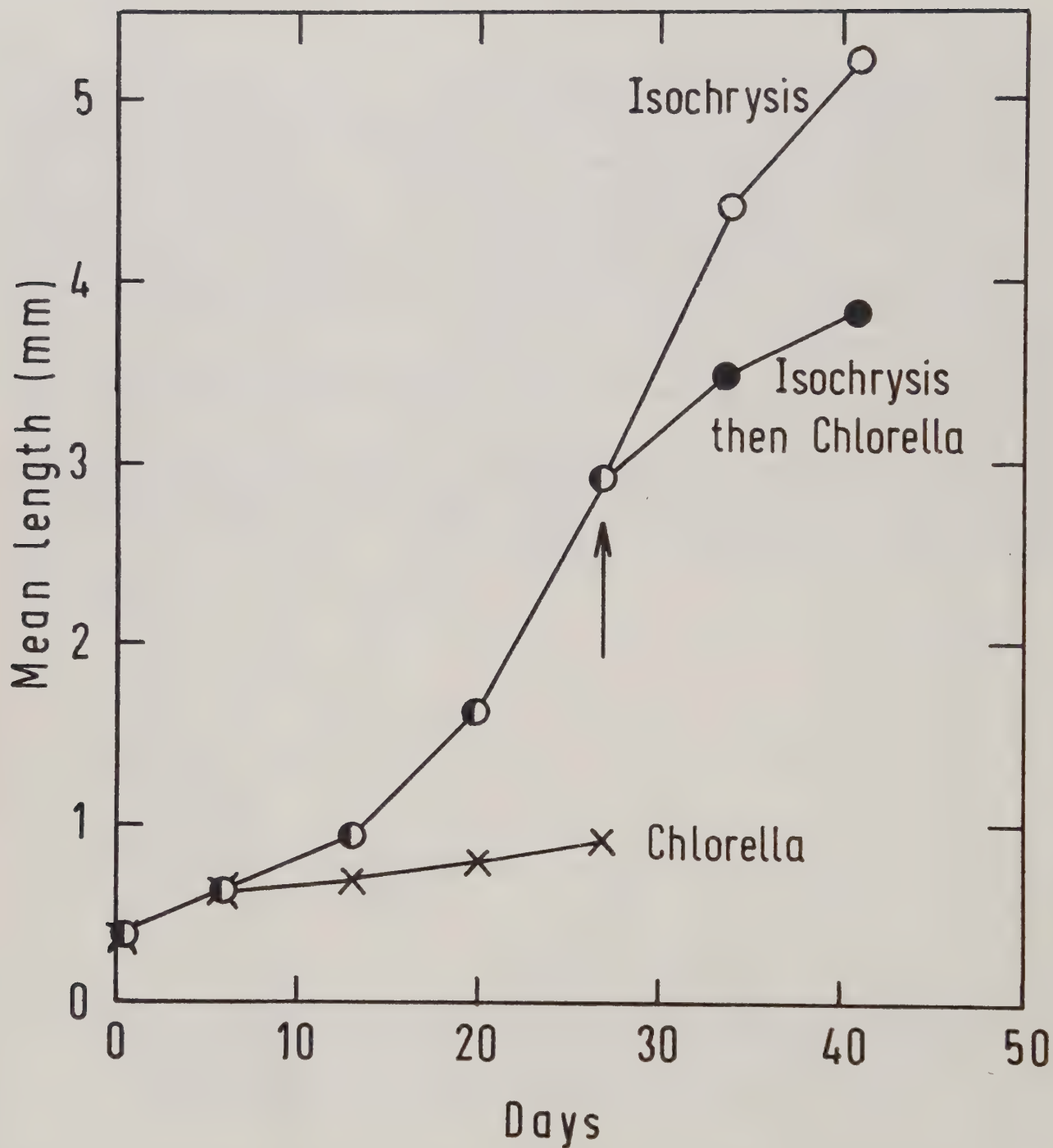


Figure 19 The growth of *Ostrea edulis* when fed on *Chlorella stigmatophora*, compared with that of the *Isochrysis* control: crosses fed on 10 *Chlorella* per μl , solid circles fed on *Isochrysis* for 27 days and then changed to 10 *Chlorella* per μl (arrowed).

CHLORELLA

Two species of *Chlorella* were used in this work: *C. stigmatophora* and the slightly smaller *C. autotrophica* (formerly known as *Chlorella* 580) which has been used a good deal as a food organism at Milford, U.S.A. Both grew well in culture. Members of this genus are frequently isolated from sea water, and all usually grow well in the laboratory. Because of this, numerous experiments have been made using these plants as food for bivalve larvae. The results generally have been unsatisfactory (Davis and Guillard 1958, Walne 1963). Davis and Calabrese (1964) reported that the food value of *C. autotrophica* and *Dunaliella euchlora* was apparently improved in experiments where the temperature exceeded 25°C, and suggested that these high temperatures were required for the digestive enzymes of the larvae to be able to deal with the rigid cell wall that is characteristic of *Chlorella*.

C. stigmatophora was fed to five species of bivalves from four genera (Figures 10, 13, 14 and App. Tables 21, A, B, 22). In the great majority of experiments growth was poor or very poor compared with the controls. The only exceptions were two tests with *O. edulis* (Figure 10) where growth in filtered and unfiltered water was compared. This type of result, where occasionally growth is much better than usual, has been obtained with oyster larvae (Walne 1956). One experiment with *O. edulis*, where the food was changed from *Isochrysis* to *Chlorella* after 27 days (Figure 19), showed that the larger spat were also unable to utilize this food. No better results were obtained with *C. autotrophica* (App. Table 21, C, D). Clams grew very poorly whether fed in filtered or unfiltered water (Figures 13 and 14).

In one experiment samples of 10 juvenile *Mercenaria*, measuring 1.1 mm, were fed on 100 cells of *C. stigmatophora* per μ l for 21 days at a range of temperatures. The mean sizes at the end of the test were as follows:

Mean temperature (°C)	Mean length (mm) after 21 days	
	Unfiltered water	Filtered water
21.0	1.63	1.29
23.3	1.64	1.32
25.2	1.73	1.34
27.0	1.65	1.35

The controls, at 21°C, fed with 75 cells per μ l of *Isochrysis* in filtered water, grew to 2.24 mm. These results did not show any enhanced growth rate with increased temperature when the food was *Chlorella*, and we must conclude that in general this is an unsatisfactory food for bivalves.

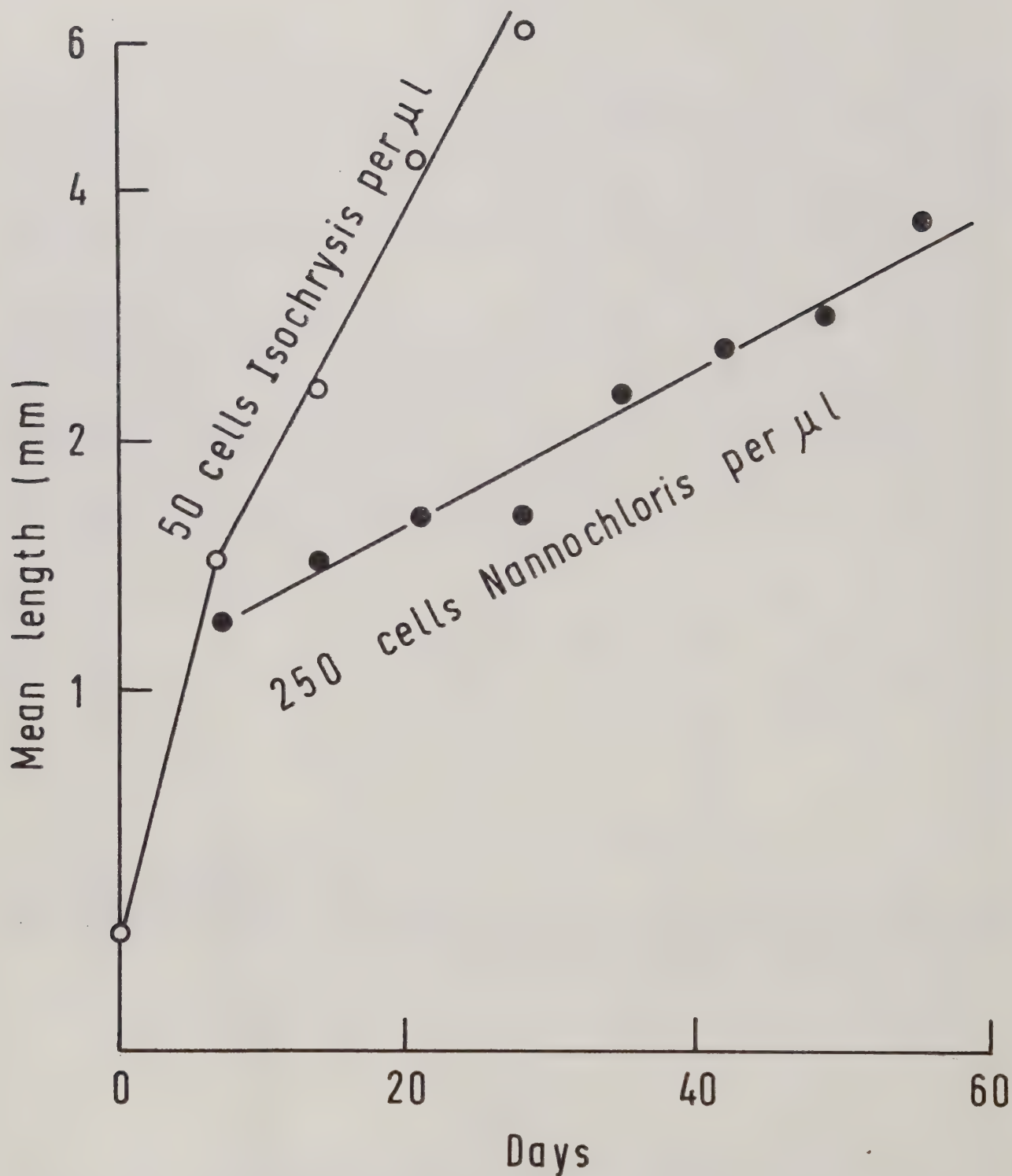


Figure 20 The growth of *Ostrea edulis* when fed on *Nannochloris atomus*, shown as the mean shell length of two experiments plotted on a $\log_e$ scale against time, compared with that of the *Isochrysis* control.

NANNOCHLORIS ATOMUS

This is a small non-motile alga which is only a little larger than the smallest species tested (*Micromonas*). The three series of experiments with *O. edulis* covered the range of 50 to 1 000 cells per μl (App. Table 23, A, B, C). Reasonably good growth was obtained and at 500 cells per μl it was about half that in the *Isochrysis* control. In one longer trial oyster spat were fed with 250 cells per μl for 56 days; the growth rate was similar throughout the period, although considerably less than in the *Isochrysis* control (Figure 20). The growth rate was not improved by feeding with *Nannochloris* in unfiltered water (Figure 10). Growth was poor in the trials made with the clam, *M. mercenaria* (Figure 13, App. Table 23, D).

It appears that *Nannochloris* has a reasonable nutritive value for oysters, but high densities of cells are required.

BRACHIOMONAS SUB-MARINA

Very poor results were obtained with this species. It failed to support growth of oyster spat (see App. Table 25, C) and in another experiment when concentrations ranging from 2.5 to 10 cells per μl were fed to *Mercenaria*, of a mean length of 1.6 mm, no detectable growth was found in 14 days in either filtered or unfiltered water although the control fed with *Tetraselmis* grew to 2.5 mm.

Xanthophyceae

OLISTHODISCUS SP.

Contrasting results were obtained when this species was fed to *Ostrea edulis* and *Mercenaria mercenaria*. Growth was poor with oysters, but more substantial growth was obtained with clams fed with concentrations between 1 and 7.5 cells per μ l (App. Table 24). One test with clams was continued for 91 days (App. Table 24, E) and although growth was reduced in the last half of the period the total obtained was only a little less than in the *Isochrysis* control.

Cryptophyceae

CRYPTOMONAS SP. (*CRYPTOMONAD* 5A, MILFORD)

The Cryptophyceae do not usually form dense cultures and are therefore difficult to use in food tests. This species is exceptional for the group because it grows well in the continuous culture apparatus.

Only moderate growth was obtained when it was fed to *O. edulis* spat in filtered water, but the results obtained when it was fed in unfiltered water suggested that it was utilized to some extent (App. Table 25, A, B). In one experiment where it was fed to *Mercenaria* at 75 cells per μ l (Figure 14) good results were obtained in filtered water, and some additional growth was obtained using unfiltered water.

Bacillariophyceae

PHAEODACTYLUM TRICORNUTUM

This species is a well-known laboratory plant which was originally isolated at Plymouth over 50 years ago (Wilson 1946). Its systematic position has for long been uncertain, but the balance of evidence (Lewin 1958) suggests that it is an aberrant diatom characterized by the possession of only one silicified valve which may not always be present. As it grows readily in continuous culture, and is a popular laboratory plant, a considerable number of experiments were made to test its food value. These were disappointing for oysters but better results were obtained with clams and mussels.

Three series of experiments were made with *Ostrea edulis* at various cell densities in the range of 5 to 150 cells per μl (App. Table 26). In all of them growth was less than in the *Isochrysis* controls, and there was a tendency for growth to be better at the lower cell densities (Figure 21). This was also found with rather larger spat when on completion of the experiment the *Isochrysis* control from one of these series

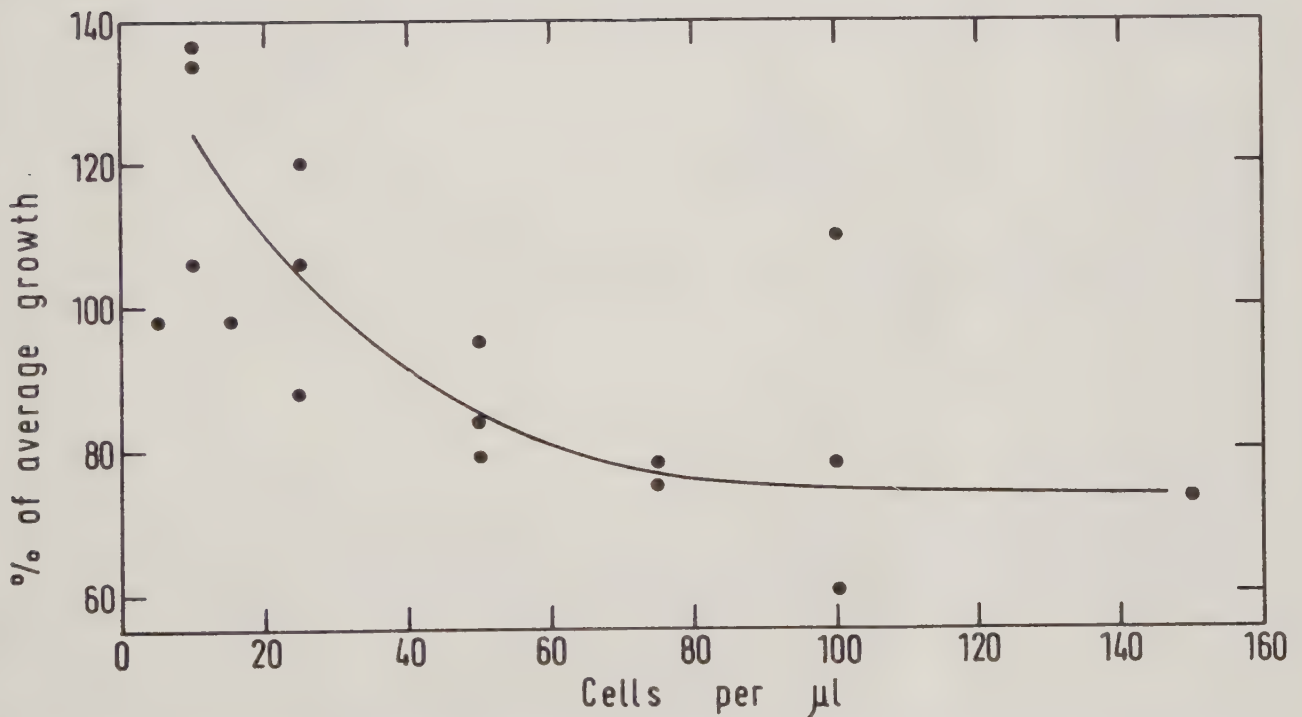


Figure 21 The growth of *Ostrea edulis* when fed on *Phaeodactylum tricornutum*, shown as the growth at different cell densities, expressed as a percentage of the mean.

was transferred to *Phaeodactylum*. This control comprised three separate plates and after the oysters on these had been fed on *Isochrysis* for 25 days they were transferred to 10, 25 and 75 cells per μl of *Phaeodactylum*. The mean lengths, after 21 and 56 days on this diet, were:

Concentration of <i>Phaeodactylum</i>	Mean length (mm) on day		
	0	21	56
10 cells per μl	2.34	5.20	7.70
25 cells per μl	2.40	3.95	4.69
75 cells per μl	2.52	3.57	3.32

Clearly, considerable growth was obtained with 10 cells per μl , although the rate steadily declined during the experiment, but higher densities of food were unsuitable.

In another test, spat with an average length of 13.6 mm which had been grown on *Isochrysis* for 38 days were transferred to 25 cells of *Phaeodactylum* per μl for 20 days. Negligible shell growth occurred. At the beginning and end the dry weight of the meat and the upper valve of the shell were measured. Before

feeding on *Phaeodactylum*, the ratio $\frac{\text{dry meat}}{\text{dry shell}}$ was 0.098. At the end of the test it was 0.042. This change shows that there was a substantial loss of meat, relative to the shell, in this 20-day period.

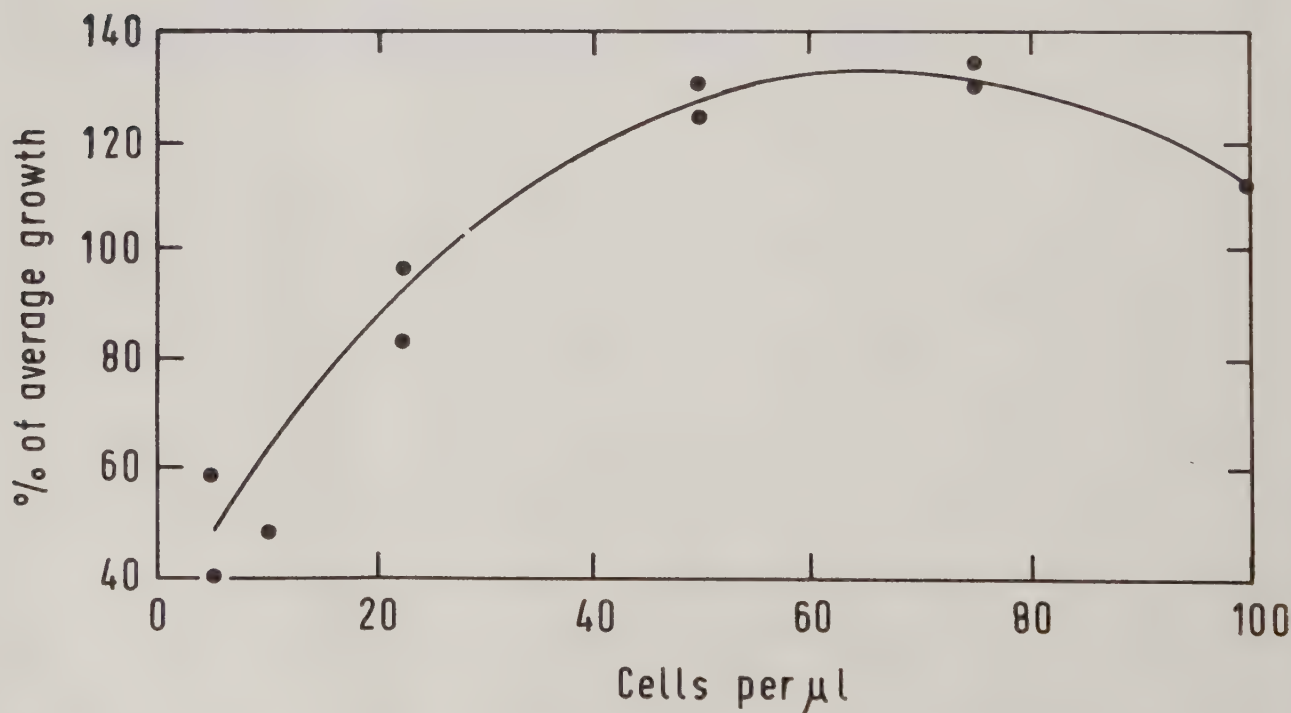


Figure 22 The growth of *Ostrea edulis* on *Skeletonema costatum*, shown as the growth at different cell densities in two experiments, expressed as a percentage of the mean.

In one trial made with unfiltered water (Figure 10) the low concentration of *Phaeodactylum* was utilized to some extent. A noticeable feature of the experiments where oyster spat were fed on the higher concentrations was the abnormal shape to which the spat grew. Instead of maintaining an approximately circular shape, the animal became elongated due to the reduced growth of the shell adjacent to the inhalant and exhalant margins. It seems possible that the poor growth recorded in the higher cell concentrations may be due, at least in part, to the physical irritation caused by the siliceous spines of this plant.

Two series of experiments were made with clams (App. Table 27, A, B); in neither was the growth particularly good, but no evidence was seen of the higher cell densities being unfavourable. When fed in unfiltered water (Figures 13 and 14) there was more growth than in the control but not as much as with a number of other species.

Two series of experiments were made with juvenile mussels (*Mytilus edulis*) of about 2 mm in length collected from the shore. The results showed that growth was less than that obtained in the *Isochrysis* control, with the exception of one batch which was fed with 50 cells of *Phaeodactylum* per μ l (App. Table 27, C, D).

THALASSIOSIRA FLUVIATILIS

This diatom grows well in culture but a thick culture has a rather characteristic "syrupy" appearance, indicating that there are possibly considerable amounts of external metabolites present which alter the viscosity of the medium. In one experiment it was fed to oyster spat of 0.7 mm in mean length for 14 days, when the following result was obtained:

Food	Water	Mean length (mm)
0.5 cells of <i>Thalassiosira</i> per μ l	Filtered	0.83
1.0 cells of <i>Thalassiosira</i> per μ l	Filtered	0.76
0.5 cells of <i>Thalassiosira</i> per μ l	Unfiltered	0.78
75 cells of <i>Isochrysis</i> per μ l	Filtered	3.13
75 cells of <i>Isochrysis</i> per μ l	Unfiltered	4.37
None	Unfiltered	2.56

It was apparently a very poor food and in addition had an inhibitory effect on growth when fed in the presence of unfiltered water.

SKELETONEMA COSTATUM

Only occasionally would this species grow into thriving cultures, so only a few abbreviated experiments could be made. Good growth was obtained in two series of experiments with *Ostrea edulis* (App. Table 28, A, B) a high degree of similarity of response being obtained in both (Figure 22). The most favourable concentration was in the range 50 to 75 cells per μ l, where the growth was about the same as in the *Isochrysis* controls. Good growth was also obtained in one experiment with clams (App. Table 28, C); on this occasion the *Isochrysis* control did not do well.

Skeletonema is apparently a good food for oysters and clams and is one of the few examples of a good food which is known to be abundant at times in inshore waters. Nelson (1947) has suggested that this species of diatom is a particularly useful food for oysters in Delaware Bay.

CHAETOCEROS CALCITRANS

A culture of this diatom was obtained from Professor Imai towards the end of this work. It grows very well in culture and supports excellent growth of oyster spat (App. Table 29). It is now frequently used as a food for both oyster larvae and oyster spat in this laboratory.

Table 5 The growth of *Ostrea edulis* spat when fed on various foods compared with the growth of controls on *Isochrysis*. The index has been calculated by dividing, for each experimental series, the largest mean size in the series at the measurement made on the nearest to 21 days from the commencement of the test, by the mean size of the control on that day

Species	Index of food value	
	Individual experiments	Average
<i>Monochrysis lutherii</i>	1.70, 1.03	1.36
<i>Chaetoceros calcitrans</i>	1.28	1.28
<i>Tetraselmis suecica</i>	1.42, 1.06, 1.12	1.20
<i>Skeletonema costatum</i>	0.93, 1.09	1.01
<i>Isochrysis galbana</i>	—	1.00
<i>Dicrateria inornata</i>	0.85, 1.04	0.94
<i>Cryptomonas</i> sp.	0.54, 0.74	0.64
<i>Cricosphaera carterae</i>	0.41, 0.83, 0.61	0.62
<i>Chlorella stigmatophora</i>	0.65, 0.56	0.60
<i>Phaeodactylum tricornutum</i>	0.62, 0.43, 0.73	0.59
<i>Olisthodiscus</i> sp.	0.47, 0.71	0.56
<i>Nannochloris atomus</i>	0.60, 0.60, 0.41	0.54
<i>Chlorella autotrophica</i>	0.37, 0.66	0.52
<i>Pavlova gyrans</i>	0.69, 0.32	0.50
<i>Micromonas pusilla</i>	0.50, 0.40, 0.42	0.44
<i>Dunaliella euchlora</i>	0.40	0.40
<i>Dunaliella tertiolecta</i>	0.36, 0.42	0.39
<i>Chlamydomonas coccoides</i>	0.26, 0.33	0.30

DISCUSSION

It is clear from the data presented on the preceding pages that the amount of growth obtained varied with the different foods. Although a given alga did not always support the same rate of growth, the rate relative to the control tended to remain the same and from this it is possible to arrange the foods in an hierarchical order. This is straightforward for *Ostrea edulis*, since these experiments all started with animals of similar size and in the same stage of development, but it is less so for clams. The other species of molluscs have been less intensively examined.

A comparative series for *O. edulis* is given in Table 5. This was obtained by dividing, for each experimental series, the largest mean size in the series at the measurement made on the day nearest to 21 days from the commencement of the test, by the mean size of the control (usually *Isochrysis*) on that day. Considering the approximations that this procedure involves, the indices for any one food agree closely. Five genera gave results which suggested a good food value similar to that of *Isochrysis*: *Dicrateria*, *Skeletonema*, *Tetraselmis*, *Chaetoceros* and *Monochrysis*.

With clams (*M. mercenaria*) it is more difficult to make a comparison because animals were used which differed considerably in size. An attempt has been made to calculate an index on a similar basis to that used for oysters for each food, but using the mean live weight. In those experiments where the clams were measured, the lengths have been converted to weight using the regression given on page 3. As expected the indices obtained (see Table 6) vary more than those from *O. edulis*, but there is a good deal of similarity in the hierarchical order of the species. Of the five found to be as good or better than *Isochrysis* for oysters, two, *Monochrysis* and *Dicrateria* are not so prominently good foods for clams.

Table 6 The growth of *Mercenaria mercenaria* when fed on various foods compared with the growth of controls fed on *Isochrysis* (some on *Tetraselmis*). The index has been calculated by dividing, for each experimental series, the largest mean size in the series at the measurement made on the nearest to 21 days from the commencement of the test, by the mean size of the control on that day

Species	Index of food value	
	Individual experiments	Average
<i>Skeletonema costatum</i>	3.30	3.30
<i>Pyramimonas grossii</i>	1.19	1.19
<i>Tetraselmis suecica</i>	1.17, 1.07, 1.09	1.11
<i>Isochrysis galbana</i>	—	1.00
<i>Nannochloris atomus</i>	0.95, 0.23, 1.57	0.92
<i>Olisthodiscus</i> sp.	0.72, 0.55, 0.97	0.75
<i>Micromonas pusilla</i>	0.73, 0.76	0.74
<i>Cricosphaera carterae</i>	1.10, 0.50, 0.50	0.70
<i>Dicrateria inornata</i>	0.28, 1.04, 0.70	0.67
<i>Monochrysis lutherii</i>	0.62, 0.63, 0.53	0.59
<i>Phaeodactylum tricornutum</i>	0.44	0.44
<i>Chlorella stigmatophora</i>	0.45, 0.17	0.31
<i>Chlamydomonas coccoides</i>	0.19	0.19
<i>Dunaliella tertiolecta</i>	0.14	0.14

All previous work of this type has been concerned with the larvae of bivalves, and the results summarized in Tables 5 and 6 can be compared with the results obtained in that field. In brief, it can be said that good food for juveniles is also good for larvae. *Dicrateria* and *Isochrysis* are excellent for oyster larvae (Walne 1956, 1965); *Tetraselmis* is now used extensively at Conway as a larval food (Walne and Spencer 1968). *Monochrysis* has been used extensively as a larval food (Loosanoff and Davis 1963, Millar and Scott 1967), but *Skeletonema* does not seem to have been tried. On the other hand the green forms, *Dunaliella*, *Chlamydomonas* and *Chlorella*, have generally given indifferent or variable results (Walne 1956, 1964; Davis and Guillard 1958).

Why do different algal species vary in their food value? The short answer is that for both larvae and juveniles we do not know. It has been suggested that forms with a rigid cell wall (for example, *Chlorella* and *Chlamydomonas*) are not readily assimilated, but this does not explain why the related *Dunaliella*, which has no rigid cell wall, should do badly. Gross chemical composition is unlikely to be helpful, because algae are very plastic in this respect; the balance of fat, carbohydrate and protein is strongly influenced by the culture conditions and age. Several workers have examined the amino-acid composition of algae, but recently Cowey and Corner (1966) have remarked that their own results in this field "indicate that any difference between particular algae in their value as foods for zooplankton are not likely to be explained in terms of differences in amino-acid composition".

Table 7 The amino-acid composition, expressed as μ moles amino-acid per 10 mg sample, of *M. mercenaria* fed for 21 days on two algal foods (see text)

	Composition of clams fed on		Composition of algal cultures	
	<i>Chlamydomonas</i>	<i>Tetraselmis</i>	<i>Chlamydomonas</i>	<i>Tetraselmis</i>
Cysteic acid	0.416	0.380	0.083	0.114
Methionine sulphone	0.588	0.570	0.214	0.252
Aspartic acid	2.612	2.650	1.113	0.900
Threonine	1.524	1.620	0.591	0.624
Serine	1.528	1.910	0.598	0.588
Glutamic acid	2.804	2.830	1.050	0.936
Proline	0.995	0.995	0.549	0.336
Glycine	1.780	1.928	0.988	1.020
Alanine	1.744	1.850	1.140	1.308
Valine	1.284	1.430	0.640	0.636
Isoleucine	1.188	1.130	0.410	0.408
Leucine	1.668	1.750	0.869	0.840
Tyrosine	0.604	0.530	0.229	0.216
Phenylalanine	0.776	0.720	0.389	0.396
Lysine	1.636	1.610	0.640	0.576
Histidine	0.656	0.770	0.261	0.168
Arginine	1.392	1.130	0.345	0.420

The writer recently had the opportunity to explore this approach for the food of bivalves through the kindness of Dr. P. T. Grant of the NERC Fisheries Biochemical Research Unit at Aberdeen, who arranged for some amino-acid analyses to be made in his laboratory. Samples of *Mercenaria mercenaria* were grown at Conway for 21 days on either *Tetraselmis suecica* or *Chlamydomonas coccooides*. During this time the dry meat weight of control clams increased by 143 and 52 per cent respectively. At the end of the period the clam meat, along with similarly preserved samples of *Tetraselmis* and *Chlamydomonas*, were sent to Aberdeen for analysis. The results of the analyses, given in Table 7, do not show any substantial differences in the amino-acid composition between the two foods or between the clams fed on the respective foods. There is no evidence from these data that the poorer growth obtained with *Chlamydomonas* was due to an unsatisfactory balance of its amino-acid composition, or to the inability of the clams to assimilate some essential amino-acid from this food. The reason apparently lies in either the digestibility of the protein as a whole or, perhaps more likely, in a field other than that of protein chemistry.

Although good growth has been obtained with some foods, it is probable that experiments of longer duration will show up serious shortcomings in many of them. Provasoli, Shiraishi and Lance (1959) and Shiraishi and Provasoli (1959) have found this for *Artemia* and *Tigriopus*, although in some cases it was necessary to go on for many generations before the shortcomings became apparent. Some assistance in sorting useful from worthless foods can be expected from studies on the growth obtained by feeding mixed foods. Experiments which will be reported in a later paper have shown that with a few mixtures of two species of algae a synergistic effect is obtained. For example, a mixture of *Tetraselmis* and *Isochrysis* gives better growth than either species on its own.

The data obtained in this work give some information on the most favourable concentrations of food in the water. For each experiment, the cell density at which the greatest growth was obtained has been picked out. In Figure 23, A, B this is related to the median cell volume (obtained from Table 2) of the food for oysters and for clams. These figures demonstrate that, independently of the food value, certain concentrations are preferred which can be expressed in terms of the total volume of algae per unit volume of water. Over much of the range of sizes covered this approximates to 5 000 μ^3 of algal material per μ l, but where the cell volume was $<10\mu^3$ rather lower concentrations were better.

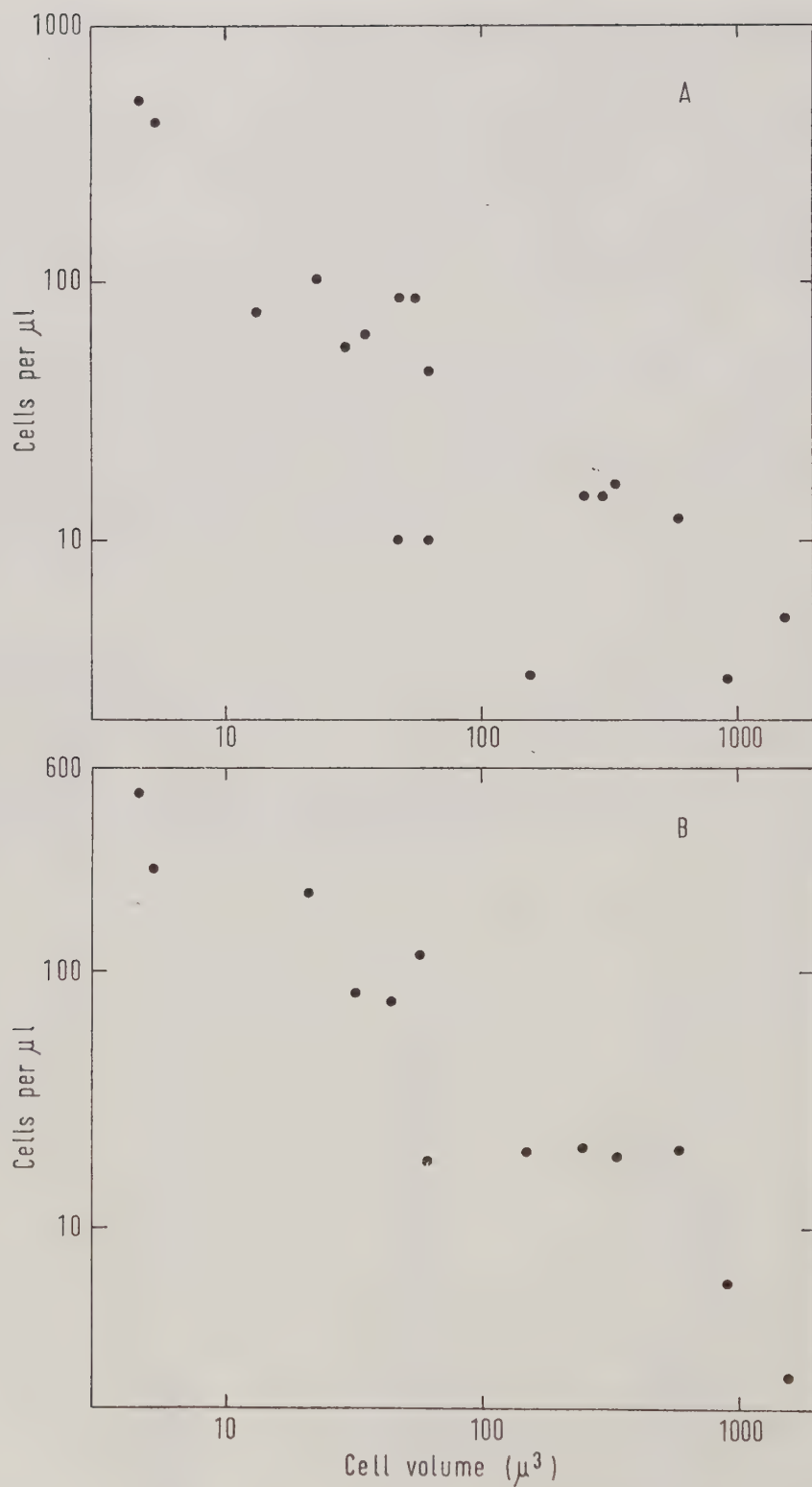


Figure 23 Relation between the lowest cell density at which maximum growth was obtained and the median cell volume for (A) *Ostrea edulis* and (B) *Mercenaria mercenaria*.

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The writer is particularly indebted for the very great assistance given during this work by his colleagues Mr. B. E. Spencer and Mr. G. J. Dean. A number of microbiologists have been very generous in sending cultures of algae.

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Appendix Table 1 The growth of *Ostrea edulis* when fed on *Dicrateria inornata* in two experiments

Food concentration (cells per μ l)	Mean length (mm) after			
	A		B	
	12 days	26 days	15 days	22 days
300 <i>D. inornata</i>	1.2	2.8	1.9	2.8
200 "	1.0	2.9	2.1	3.2
150 "	1.3	3.9	2.5	4.1
100 "	1.3	4.1	2.7	4.4
50 "	1.0	1.6	2.1	2.1
50 <i>I. galbana</i> (control)	2.1	3.4	3.5	5.2
Initial size (mm)	0.64		0.76	

Appendix Table 2 The growth of *Mercenaria mercenaria* when fed on *Dicrateria inornata* in three experiments

Food concentration (cells per μ l)	Mean length (mm) after				
	A		B		C
	14 days	21 days	21 days	42 days	63 days
300 <i>D. inornata</i>	1.06	1.21			
200 "	0.93	1.05			5.43
175 "					5.24
150 "	0.93	1.03			
125 "					5.39
100 "	0.79		3.01	3.41	
75 "			2.94	3.18	
50 "			3.08	3.35	4.59
25 "			2.82	2.83	
75 <i>I. galbana</i> (control)	1.09	1.34	4.57	6.65	5.68
Initial size (mm)	0.63		2.13		4.26

Appendix Table 3 The growth of *Ostrea edulis* when fed with *Isochrysis galbana* in six experiments

Food concentration (cells per μ l)	Mean length (mm) after					
	A	B	C	D	E	F
	47 days	47 days	42 days	37 days	26 days	21 days
150 <i>I. galbana</i>					2.40	2.31
100 "	2.39		5.88		3.01	2.37
75 "	6.10	8.46	4.60			2.61
50 "	6.80	9.18	6.60	7.82	1.94	2.54
25 "	9.66	8.50	7.84	6.88		2.76
10 "	7.49	5.19	5.51	5.48		1.22
5 "		3.87	5.56			0.87
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Initial size (mm)	0.40	0.36	0.46	0.49	0.52	0.64

Appendix Table 4 The growth of *Mercenaria mercenaria* when fed on *Isochrysis galbana*

Food concentration (cells) per μ l	Mean length (mm) after					
	A	B	C	D	E	F
	14 days	13 days	14 days	7 days	38 days	27 days
200 <i>I. galbana</i>			1.18			
150 "			1.06		4.41	7.03
100 "	0.84	0.96	1.07	1.90	4.88	7.62
75 "		0.96	0.77	1.62	4.56	7.38
50 "	0.68	0.95	0.74	1.76	4.72	7.50
25 "	0.45	0.83	0.64	1.48	4.67	7.16
10 "	0.42	0.51	0.68	1.42	4.13	6.39
5 "		0.49				
0 "				1.44		
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Initial size (mm)	0.40	0.49	0.63	1.30	2.40	4.50

Appendix Table 5 Growth of *Ostrea edulis* (A and C), *Ostrea lutaria* (B), and *Mercenaria mercenaria* (D) when fed on *Cricosphaera* 156

Food concentration (cells per μ l)	Mean length (mm) after			Mean weight (mg) after	
	A	B	C	D	
	23 days	28 days	22 days	21 days	
	FW	FW UFW	FW UFW	FW	UFW
15 <i>Cricosphaera</i> 156	1.49				
10 ,,	1.34				
7.5 ,,	1.62	1.40		75.9	
5 ,,	1.89	1.56 1.87	2.42 8.59	70.6	66.3
2.5 ,,	1.53	1.41 1.63		74.6	79.2
1 ,,	0.98	1.70 1.80		54.7	59.8
0.5 ,,		1.81 —			
0 ,,		1.05 1.77		55.2	52.3
50 <i>I. galbana</i> (control)	4.56	— —	4.68 9.63		84.7*

Initial size (mm or mg)	0.60	0.50	0.50	55	

* 20 cells *Tetraselmis* per μ l.

Note: FW = Filtered water. UFW = Unfiltered water.

Appendix Table 6 Growth of *Ostrea edulis* (A and B) and *Mercenaria mercenaria* (C) when fed on *Cricosphaera* 181

Food concentration (cells per μ l)	Mean length (mm) after		
	A	B	C
	18 days	16 days	21 days
10 <i>Cricosphaera</i> 181	1.43		0.95
7.5 "	1.56	1.46	0.92
5 "	1.49	1.68	0.93
2.5 "	1.77	1.60	0.85
1 "	1.90	1.32	0.81
0.5 "		1.08	
50 <i>I. galbana</i> (control)	2.35	2.49	0.91
Initial size (mm)	0.70	0.60	0.76

Appendix Table 7 Growth of *Ostrea edulis* (A and B), *Ostrea lutaria* (C) and *Crassostrea gigas* (D and E) when fed on *Monochrysis lutherii*. The controls were fed with: A, 50 cells *Isochrysis* per μ l; B, 100 cells *Isochrysis* per μ l; C, 2.5 cells *Tetraselmis* per μ l; D, 75 cells *Isochrysis* per μ l; E, no control

Food concentration (cells per μ l)	Mean length (mm) after						
	A	B	C		D	E	
	18 days	28 days	14 days	21 days	22 days	14 days	
						Filtered water	Unfiltered water
200 <i>M. lutherii</i>						1.24	1.90
150 "			1.44	1.58		1.12	2.30
100 "	1.80	3.09	1.55	1.67	1.87	1.37	2.20
75 "	2.01	2.13			2.00	1.60	2.32
50 "	2.56	2.33	1.49	1.55	2.51	1.44	2.49
25 "	3.03	2.93	1.57	1.59	2.10	1.10	1.78
10 "		1.34	1.35	1.35	2.12		
Control (see caption)	1.78	3.01	2.64	4.19	1.59		
Initial size (mm)	0.59	0.52	0.84		0.70	0.5	

Appendix Table 8 Growth of *Mercenaria mercenaria* when fed on *Monochrysis lutherii*

Food concentration (cells per μ l)	Mean length (mm) after			
	A	B	C	
	69 days	38 days	21 days	
			Filtered water	Unfiltered water
150 <i>M. lutherii</i>			1.75	2.00
100 ,,		5.43	1.80	2.00
75 ,,		5.05	1.73	
50 ,,	5.81	4.24	1.60	
40 ,,	5.25			
30 ,,	5.41	3.83		
20 ,,	4.66			
10 ,,	3.25			
75 <i>I. galbana</i> (control)	7.52	6.65	2.24	
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Initial size (mm)	2.13	2.08	1.11	

Appendix Table 9 Growth of *Ostrea edulis* (A and B) and *Mercenaria mercenaria* (C) when fed on *Pavlova gyrans*. The controls were fed with: A and B, 75 cells *Isochrysis galbana* per μl ; C, 20 cells *Tetraselmis suecica* per μl

Food concentration (cells per μ l)	Mean length (mm) after		Mean weight (mg) after		
	A		B	C	
	23 days	37 days*	20 days	21 days	
				Filtered water	Unfiltered water
150 <i>P. gyrans</i>			0.94	191	
125 "			1.04		
100 "			1.11	209	238
75 "	1.48	1.59	1.02	223	228
50 "	1.21	1.38		204	
25 "	0.79	0.80		182	
10 "	0.60				
5 "	0.58				
Control (see caption)	2.18	3.09	3.40		317
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Initial size (mm or mg)	0.49		0.64	120	

* After 23 days the concentrations of 25 and 50 cells per μl were increased to 100 and 150 cells per μl respectively.

Appendix Table 10 Growth of *Ostrea edulis* (A, B and C) and *Crassostrea gigas* (D and E) when fed on *Micromonas pusilla*. In A, B and C the *Isochrysis* control concentration was 50 cells per μl ; in D it was 75 cells per μl ; in E no control

Food concentration (cells per μl)	Mean length (mm) after								
	A		B		C		D	E	
	22	36	20	35	17	31	22	14 days	
	days	days	days	days	days	days	days	FW	UFW
2 000 <i>M. pusilla</i>			1.24	2.19					
1 000 „			1.22	2.20	0.86	1.45	1.26	2.18	3.86
500 „	1.30	2.10	1.25	2.05	1.03	1.36	0.94	2.12	3.10
250 „	1.04	1.47					0.92		
100 „	0.86	1.24	0.97		1.14	1.98	0.82	2.05	3.30
50 „	0.98	1.20	1.22	1.74			0.90		
0 „									3.25
Control (see caption)	2.64	3.64	2.95	6.84	2.25	4.94	1.54	—	—
Initial size (mm)	0.6		0.3		0.3		0.7	1.6	

Note: FW = Filtered water. UFW = Unfiltered water.

Appendix Table 11 Growth of *Mercenaria mercenaria* (A and B) when fed on *Micromonas pusilla* and of *Ostrea edulis* (C) when fed on *Micromonas squamata*

Food concentration (cells per μ l)	Mean length (mm) after				Food concentration (cells per μ l)	Mean length (mm) after	
	A		B				C
	14 days	21 days	14 days	21 days			14 days
2 000 <i>M. pusilla</i>	0.84	0.96	1.66	1.70	470 <i>M. squamata</i>	2.33	
1 000 ,,	0.98	1.01	1.93	2.08	350 ,,	2.08	
500 ,,	1.07	1.41	1.92	2.08	236 ,,	2.55	
100 ,,	0.84	0.91	1.42	1.48	177 ,,	2.37	
50 ,,	0.79	0.86	1.30	1.32	118 ,,	2.31	
					59 ,,	2.63	
					24 ,,	2.66	
75 <i>I. galbana</i> (control)	1.16	1.58	1.96	2.40	2.5 <i>T. suecica</i> (control)	4.08	
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Initial size (mm)	0.55		1.06		Initial size (mm)	0.89	

Appendix Table 12 Growth of *Ostrea edulis* when fed on *Tetraselmis suecica*

Food concentration (cells per μ l)	Mean length (mm) after					
	A		B		C	
	26 days	33 days	16 days	23 days	21 days	29 days
30 <i>T. suecica</i>	3.65	5.17	2.52	3.72		
20 "	3.89	5.37	2.58	3.74		
15 "	3.81	5.07	2.64	3.38		
10 "	4.17	5.08	2.64	3.44	3.83	6.22
7.5 "					3.58	5.33
5 "	2.07	2.41	1.43	1.54	1.45	1.63
2.5 "	1.27	1.27	0.93	0.93	0.90	
1 "					0.68	
50 <i>I. galbana</i> (control)	3.10	4.20	2.46	3.52	3.39	5.28
Initial size (mm)	0.60		0.73		0.49	

Appendix Table 13 Growth of *Ostrea edulis* when fed on *Tetraselmis* sp. A: *T. tetrahele*, B and C: *T. inconspicua*, D: *T. chui*

Food concentration (cells per μ l)	Mean length (mm) after			
	A	B	C	D
	21 days	24 days	21 days	14 days
30 <i>Tetraselmis</i> sp.	3.09	2.67	2.60	2.64
20 „	4.21	2.90	3.43	3.79
15 „	4.33	2.92	4.70	3.82
10 „	1.98	2.31	1.99	3.40
7.5 „			1.66	
5 „	0.79	1.64	0.98	3.95
2.5 „				3.48
1 „				2.93
Initial size (mm)	0.48	0.59	0.52	1.03

Appendix Table 14 Growth of *Ostrea edulis* when fed on 10 cells per μ l of *Tetraselmis* in filtered and unfiltered sea water for 21 days

Food species	Mean length (mm)					
	Filtered sea water			Unfiltered sea water		
	A	B	C	A	B	C
<i>T. inconspicua</i>	2.02	1.99				8.12
<i>T. suecica</i>	1.11	3.25	4.21	7.02	6.56	8.38
<i>T. tetrahele</i>	1.28	2.18				9.95
Initial size (mm)	0.52	0.52	0.81	0.52	0.52	0.81

Appendix Table 15 Growth of *Crassostrea gigas* (A and B) and *Ostrea lutaria* (C) when fed on *Tetraselmis suecica*

Food concentration (cells per μ l)	Mean length (mm) after				
	A	B		C	
	26 days	14 days		14 days	
		Filtered water	Unfiltered water	Filtered water	Unfiltered water
50 <i>T. suecica</i>	2.19				
30 "	2.39	1.34	1.97	1.21	
20 "	2.55	2.28	2.63	1.21	
15 "		2.09	3.25	1.29	
10 "	2.58	2.70	3.69	1.43	
5 "	2.10	2.81	3.05	1.45	
2.5 "		2.48	3.02	1.56	2.20
0 "					1.52
100 <i>I. galbana</i>	1.52				
Initial size (mm)	0.54	0.50		0.52	

Appendix Table 16 Growth of *Mercenaria mercenaria* when fed on *Tetraselmis suecica*. The *Isochrysis* control concentration was 100 cells per μl in A, and 75 cells per μl in B, C and D

Food concentration (cells per μl)	Mean length (mm) after				Mean weight (mg) after			
	A		B		C		D	
	14 days	21 days	14 days		35 days	49 days	14 days	28 days
			Filtered water	Unfiltered water				
50 <i>T. suecica</i>					29.8	31.0		
35 "					35.4	37.3		
25 "					34.4	35.1	89.9	112.5
20 "	0.88	1.10						
15 "	0.93	1.06	2.53	2.83	34.4	35.3	92.4	120.6
10 "	0.86	1.00	1.92	2.40			79.8	86.2
5 "	0.68	0.69	1.70	2.24			43.8	49.3
2.5 "	0.71	0.71	1.60	1.77				
0 "			1.71	1.64				
<i>I. galbana</i>	0.99	1.07	1.82	2.62	30.0	35.0	77.6	97.1
Initial size (mm)	0.6		1.50		18.5		36.0	

Appendix Table 17 Growth of *Mercenaria mercenaria* when fed on three species of *Tetraselmis* for 21 days. A: *T. tetrahele*; B: *T. inconspicua*; C: *T. suecica*. (Initial size 0.75 mm)

Food concentration (cells per μl)	Mean length (mm) after					
	A		B		C	
	14 days	21 days	14 days	21 days	14 days	21 days
30 <i>Tetraselmis</i>	1.66	2.20	1.55	1.87	1.81	2.29
20 "	1.73	2.24	1.46	1.86	1.71	2.16
15 "	1.43	1.68	1.31	1.59	1.56	2.09
10 "	1.18	1.50	1.17	1.50	1.22	1.66
5 "	0.92	0.95	0.85	0.92	0.85	0.88
2.5 "	0.82	0.82	0.82	0.84	0.82	0.84
75 <i>I. galbana</i> (control)	0.94	0.96	—	—	—	—

Appendix Table 18 Growth of *Ostrea edulis* when fed on *Dunaliella tertiolecta* (A and B) and *Dunaliella euchlora* (C). The *Isochrysis* controls were fed with 75 cells per μl in A, 50 cells per μl in B, and 100 cells per μl in C

Food concentration (cells per μl)	Mean length (mm) after		
	A	B	C
	21 days	17 days	28 days
50 <i>Dunaliella</i>	0.62		
40 "	0.62		0.92
30 "	0.59	0.74	0.85
20 "	0.65	0.79	0.83
10 "	0.60	0.78	1.01
5 "		0.75	1.16
Control (see captain)	1.80	1.86	3.01
Initial size (mm)	0.30	0.60	0.52

Appendix Table 19 Growth of *Ostrea edulis* (A, B and C), *Ostrea lutaria* (D), and *Crassostrea gigas* (E) when fed on *Chlamydomonas coccoides*. In D and E the controls were fed on 2.5 cells of *Tetraselmis* per μl

Food concentration (cells per μl)		Mean length (mm) after							
		A	B	C		D		E	
		26 days	29 days	21 days		28 days		14 days	
				FW	UFW	FW	UFW	FW	UFW
30	<i>C. coccoides</i>	0.94	0.81					0.80	1.45
20	„	0.90	0.89					1.24	
15	„					1.20			
10	„	1.36	1.18	1.17		1.29		1.03	2.02
7.5	„			0.95	3.42				
5	„	1.38	1.52	1.17		1.10	2.01	0.83	
2.5	„	0.97	1.68	1.20	3.74	1.13	2.02		1.71
1	„					1.05	1.98		
0	„					1.05	1.77		
25	<i>I. galbana</i>	6.24					5.34		2.57
50	„	8.05		3.68					
Initial size (mm)		0.44	0.55	0.61		0.58		0.50	

Note: FW = Filtered water. UFW = Unfiltered water.

Appendix Table 20 Growth of *Mercenaria mercenaria* when fed on *Chlamydomonas coccoides*. The controls were fed on 75 cells of *Isochrysis galbana* per μl in A, and on 20 cells of *Tetraselmis suecica* per μl in B

Food concentration (cells per μl)	Mean length (mm) after		Mean weight (mg) after	
	A		B	
	28 days		21 days	
			Filtered water	Unfiltered water
40 <i>C. coccoides</i>			70.5	83.3
30 "	1.88			
20 "	1.91		75.1	87.7
10 "	1.87		78.7	
5 "	1.89		57.2	72.4
2.5 "	1.94			
0 "				52.3
Control (see caption)	3.66			84.7
Initial size (mm)	1.84		56.4	

Appendix Table 21 Growth of *Ostrea edulis* when fed on *Chlorella stigmatophora* (A and B) and *Chlorella autotrophica* (C and D); in D the control was 10 cells per μl *Tetraselmis suecica*

Food concentration (cells per μl)	Mean length (mm) after			
	A	B	C	D
	27 days	25 days	26 days	19 days
100 <i>C. stigmatophora</i>	1.32	1.17	1.05	1.18
75 "	1.27	1.09	1.08	1.19
50 "	1.17		0.94	1.22
25 "	1.17	1.06	0.88	0.98
10 "	1.35	0.99	0.76	
75 <i>I. galbana</i> (control)	2.50	2.72	3.01	1.85
Initial size (mm)	0.40	0.40	0.52	0.57

Appendix Table 22 Growth of *Crassostrea gigas* (A), *Ostrea lutaria* (B), and *Mytilus edulis* (C) when fed on *Chlorella stigmatophora*. In C the controls were fed on 50 cells of *Isochrysis galbana* per μl

Food concentration (cells per μl)	Mean length (mm) after				
	A		B		C
	14 days		14 days		28 days
	Filtered water	Unfiltered water	Filtered water	Unfiltered water	
125 <i>C. stigmatophora</i>	0.62	1.00	1.00		
100 "	0.63	0.88	0.92		3.70
75 "	0.64	1.23			
50 "	0.54	1.13	1.03	1.64	3.30
25 "	0.67		1.04		3.27
10 "	0.82	1.39	1.04	1.76	3.29
2.5 <i>Tetraselmis</i> (control)		3.70		2.25	6.01
Initial size (mm)	0.50		0.80		2.84

Appendix Table 23 Growth of *Ostrea edulis* (A, B and C) and *Mercenaria mercenaria* (D) when fed on *Nannochloris atomus*

Food concentration (cells per μl)	Mean length (mm) after						
	A		B		C		D
	26 days	42 days	27 days	41 days	28 days	56 days	28 days
1 000 <i>N. atomus</i>	3.82		2.96				2.09
500 "	3.49	4.25	3.16	5.05	2.08	4.17	2.12
250 "	2.64	2.97	2.33	3.20	1.67	3.66	2.20
100 "	1.58		1.30				2.03
50 "	1.31		1.05				2.01
25 <i>I. galbana</i>	9.16		6.40		3.49		
50 "	10.35		7.00		6.16		4.10
Initial size (mm)	0.38		0.37		0.54		1.88

Appendix Table 24 Growth of *Ostrea edulis* (A and B) and *Mercenaria mercenaria* (C, D and E) when fed on *Olithodiscus* sp. For clams, the controls were fed with 75 cells of *Isochrysis* per μ l

Food concentration (cells per μ l)	Mean length (mm) after						Mean weight (mg) after	
	A	B	C		D		E	
	14 days	14 days	21 days	27 days	43 days	52 days	49 days	91 days
20 <i>Olithodiscus</i> sp.	0.75	0.78						
10 "	0.85	0.96						
7.5 "	0.78	0.84					39.5	45.8
5 "	0.84	0.96					41.6	47.8
2.5 "	0.88	0.86					40.0	47.4
1 "			3.03	3.51	4.00	5.05	22.4	
0.75 "			2.96	3.34				
0.5 "			2.31	2.38	2.32			
0.25 "			1.84		2.12			
0.1 "			1.88					
50 <i>I. galbana</i> (control)	1.86	1.38	3.40	3.94	7.10	7.66	35.0	54.6
Initial size (mm)	0.60	0.75	1.86		2.04		18.8	

Appendix Table 25 Growth of *Ostrea edulis* when fed on *Cryptomonas* (A and B), and *Brachiomonas sub-marina* (C)

Food concentration (cells per μ l)	Mean length (mm) after				
	A		B		C
	21 days		21 days		15 days
	Filtered water	Unfiltered water	Filtered water	Unfiltered water	
100 <i>Cryptomonas</i> sp.	0.95		0.66		
75 "	1.05		0.66		
50 "	1.10	2.00	0.66	1.45	
25 "	1.52	5.02	0.71	2.20	
10 "	1.94		0.78		
0 "		3.87		1.18	
75 <i>I. galbana</i> (control)	3.51	7.01	1.05	3.80	
10 <i>B. sub-marina</i>					0.89
7.5 "					0.92
5.0 "					0.96
2.5 "					0.95
1.0 "					1.09
50 <i>I. galbana</i> (control)					2.56
Initial size (mm)	0.47		0.36		0.79

Appendix Table 26 Growth of *Ostrea edulis* when fed on *Phaeodactylum tricornutum*

Food concentration (cells per μ l)	Mean length (mm) after				
	A		B		C
	17 days	40 days	21 days	35 days	25 days
150 <i>P. tricornutum</i>			1.11	1.01	
100 „	1.11	1.16	1.56	1.55	0.87
75 „	1.35	1.39			0.87
50 „	1.49	1.58	1.33	1.37	0.88
25 „	1.35	2.34	1.12	1.29	1.19
15 „	1.45	2.07			
10 „	1.62	3.41	1.31	1.65	1.53
5 „	1.28	2.45			
50 <i>I. galbana</i> (control)	2.63	7.82	3.69	7.77	2.42
Initial size (mm)	0.49		0.35		0.30

Appendix Table 27 Growth of *Mercenaria mercenaria* (A and B) and *Mytilus edulis* (C and D) when fed on *Phaeodactylum tricornutum*. In series A the clams were initially 2.4 mm in size when selected and were fed on *Isochrysis* for 38 days and then changed to the same cell densities of *Phaeodactylum*

Food concentration (cells per μ l)	Mean length (mm) after					
	A		B	C		D
	38 days	79 days	28 days	17 days	38 days	28 days
150 <i>P. tricornutum</i>	4.41	5.52		3.57	3.67	
100 „	5.12	5.94		3.42	3.73	3.87
75 „	4.82	5.61		3.40	3.62	
50 „			3.70	3.59	4.52	4.26
25 „	4.98	6.32	4.11	3.46	3.72	3.80
10 „	4.37	6.04	2.85	3.43	3.88	3.66
5 „			2.72			
50 <i>I. galbana</i> (control)	4.86	7.24	5.40	3.61	4.08	6.01
Initial size (mm)	See caption		2.78	2.90		2.79

Appendix Table 28 Growth of *Ostrea edulis* (A and B) and *Mercenaria mercenaria* (C) when fed on *Skeletonema costatum*

Food concentration (cells per μ l)	Mean length (mm) after		
	A	B	C
	16 days	9 days	21 days
100 <i>S. costatum</i>		1.18	1.79
75 "	2.56	1.34	1.58
50 "	2.50	1.28	1.36
25 "	1.86	1.11	1.07
10 "	1.13		1.10
5 "	0.76	0.60	1.13
50 <i>I. galbana</i>	2.80	1.21	
100 "			1.20
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Initial size (mm)	0.60	0.49	1.05

Appendix Table 29 Growth of *Ostrea edulis* when fed on *Chaetoceros calcitrans*. In experiment B the control was damaged

Food concentration (cells per μ l)	Mean length (mm) after	
	A	B
	19 days	21 days
200 <i>C. calcitrans</i>	1.99	2.23
150 "	2.02	3.50
100 "	2.76	3.36
50 "	3.08	3.16
25 "	3.15	2.92
10 <i>Tetraselmis</i> (control)	1.85	
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Initial size (mm)	0.63	0.66